SLEEP REGULATION AND RELATIONSHIP BETWEEN SLEEP AND MEMORY IN RODENTS

Dissertation

zur

Erlangung der naturwissenschaftlichen Doktorwürde
(Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

von

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Zürich, 2006

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Acknowledgements

This thesis was performed at the Institute of Pharmacology and Toxicology of the University of Zurich.
First of all I want to thank the Head of the Section of the Psychopharmacology and Sleep Research Prof. Alexander Borbély for giving me the opportunity to work on this project in his laboratory.
I would like to especially thank my supervisor Prof. Irene Tobler for her continuous support during the thesis, for introducing me to the world of science and for teaching me all aspects of research, including the design of experiments, data analysis, interpretation of results and writing of publications.
I thank Prof. Rüdiger Wehner for accepting me as a Ph.D. student at the MNF of the University of Zurich and for his supervising.
I am thankful to PD Dr. Peter Achermann for introducing me into signal analysis. Special thanks to Dr. Tom de Boer for the introduction into methods of sleep research, his constant help with programming and statistical analysis.
I would like to thank Dr. Peter Meerlo and Dr. Florence Crestani for a fruitful collaboration that resulted in two publications.
I thank Drs. Thomas Graf and Roland Dürr for development of the recording systems and their help in solving computer problems.
I would like to thank Charly Wüthrich, Jacques Kaufmann, Jörk Pischke and Harald Osswald for technical support.
I am thankful to Yvonne Maeder for handling all administrative and infrastructural problems and questions.
I thank present and former members of the group for their constant support and help during the experiments, especially the members of the animal laboratory Dr. Vladislav Vyazovskiy, Dr. Raphaëlle Winsky-Sommerer, Esther Wigger and Monique Wessely.
I am thankful to my family for their support and understanding.

Summary

The general aims of this thesis were to gain deeper insights into the regulation of sleep under different physiological conditions in rodents and thereby to understand the functions of sleep.

In contrast to a general belief, it has been suggested that Djungarian hamsters incur a sleep deficit during an episode of daily torpor and that sleep following such a torpor episode is homeostatically regulated. To examine homeostatic properties of sleep following torpor, Djungarian hamsters were subjected to partial non-rapid eye movement (NREM) sleep deprivation immediately after emergence from an episode of daily torpor. For the comparison, on a different day the same animals were subjected to 4 h total sleep deprivation (SD) followed by partial NREM sleep deprivation. The results showed that partial NREM sleep deprivation allowed sleep pressure to remain at a high level after both SD and daily torpor, which is in agreement with the two-process model of sleep regulation. Slow-wave activity (SWA) in NREM sleep started decreasing only when recovery was allowed, and did not differ between torpor and SD conditions, indicating that sleep after daily torpor is homeostatically regulated.

To investigate endogenous properties of seasonality (winter-summer), seasonal changes in sleep were investigated in Djungarian hamsters invariably maintained at low ambient temperature and in a short photoperiod. Despite constant environmental conditions, in spring the animals changed their physiology from winter to summer type. The distribution of sleep and wakefulness differed between hamsters that exhibited summer or winter physiology. The light-dark distribution of vigilance states showed a clear preference for sleep during the light period in animals exhibiting physiological traits that are typical for summer. EEG SWA in NREM sleep was evenly distributed over the light-dark period in winter, but decreased in the course of light period and increased during the dark period in summer. These data provide evidence for the existence of an endogenous clock that triggers seasonal adaptations in photoperiodic animals.

It has been suggested that sleep is involved in memory consolidation and retrieval in humans and animals. In the present thesis the relationship between sleep and memory was studied in an object recognition task in Djungarian hamsters, which were either subjected to SD or had experienced an episode of spontaneous daily torpor after the acquisition phase. SD resulted in familiarity and recency memory impairment and in a deficit in the complex spatial scene retrieval. Daily torpor also induced a deficit in the complex scene retrieval but less than SD. The results indicate that lack of sleep or sensory stimulation associated with waking experiences may result in memory impairment. Therefore, in hamsters sleep may either actively facilitate memory consolidation or provide optimal conditions of non-interference for memory consolidation.

To further elucidate the role of sleep in memory consolidation, the importance of the timing of sleep during the consolidation period was investigated in an object recognition task. These experiments were performed in mice, because such tests are widely applied in mice bred in laboratories. Moreover, a detailed analysis of sleep and the effects of SD were reported for the OF1 strain we used. Animals were subjected to 6 h SD either immediately

after the acquisition phase of the task or 6 h later. Only mice subjected to SD immediately after acquisition failed to discriminate between a novel object and two familiar objects during the test phase. In contrast, the delayed SD enabling memory consolidation during the 6 h, when the mice were allowed to sleep, had no detrimental effect on memory. Therefore, the loss of sleep or the additional activity of the mice during the SD impaired recognition memory retrieval, but only when they occurred immediately after acquisition.

In summary, the results demonstrate that sleep after daily torpor is homeostatically regulated, that there are endogenous seasonal aspects of sleep regulation and that sleep might be critically involved in recognition memory consolidation in rodents.

Zusammenfassung

Das Hauptziel der vorliegenden Dissertation war es, einen tieferen Einblick in die Regulation des Schlafes in Nagetieren unter verschiedenen physiologischen Bedingungen zu erlangen und somit die Funktion des Schlafes besser zu verstehen.

Entgegen der vorherrschenden Meinung gingen wir davon aus, dass Djungarische Hamster während ihrer Tagestorpor-Episoden ein Schlafdefizit akkumulieren und dass der auf solche Episoden folgende Schlaf homeostatisch reguliert ist. Um den auf Torpor folgenden Schlaf auf seine homeostatischen Eigenschaften hin zu untersuchen, wurden Djungarische Hamster sofort nach dem Aufwachen aus einer Tagestorpor-Episode einem teilweisen non-REM (NREM) Schlaf-Entzug unterzogen. Zum Vergleich wurden dieselben Tiere an einem anderen Tag einem vierstündigen vollständigen Schlafentzug unterzogen, gefolgt von einem teilweisen NREM-Schlaf-Entzug. Die Ergebnisse zeigten, dass der partielle NREM-Schlafentzug den Schlafdruck sowohl nach einer Torporepisode als auch nach vollständigem Schlafentzug auf einem hohen Niveau aufrechterhält. Diese Beobachtung stimmt mit dem Zwei-Prozess-Modell der Schlafregulation überein. Die langsamwellige Aktivität (Slow wave activity, SWA) während des NREM-Schlafs nahm sowohl in der Torpor- als auch in der Schlafentzugsbedingung erst in der Erholungsphase ab. Dieses Resultat unterstützt die Annahme, dass der Schlaf nach einer Tagestorpor-Episode homeostatisch reguliert ist.

Um den Einfluss von jahreszeitlichen Veränderungen (von Winter zu Sommer) auf den Schlaf zu untersuchen, wurden Djungarische Hamster bei einer tiefen Umgebungstemperatur und einer kurzen Photoperiode gehalten. Trotz dieser konstanten äusseren Bedingungen vollzogen die Tiere im Frühling einen Wechsel von einer typischen Winter- zu einer typischen Sommer-Physiologie. Die tägliche zeitliche Verteilung der Schlaf- und Wach-Zustände unterschied sich zwischen Hamstern mit winter-typischer und sommer-typischer Physiologie, wobei Schlaf während der Lichtperiode vor allem in Tieren beobachtet werden konnte, die einen Sommer-typischen physiologischen Zustand aufwiesen. Die SWA im NREM-Schlaf war im Winter über 24 Stunden regelmässig verteilt, im Sommer dagegen sank sie während der Hellperiode und stieg während der Dunkelperiode. Diese Ergebnisse liefern einen Beleg für die Existenz eines endogenen Zeitgebers, der jahreszeitbedingte Adaptationen in photoperiodischen Tieren auslöst.

Es wird vermutet, dass Schlaf bei Menschen und Tieren an der Gedächtnis-Konsolidierung und am Abruf von Gedächtnisinhalten beteiligt ist. Mittels eines Objekterkennungstests wurde in der vorliegenden Arbeit die Beziehung zwischen Schlaf und Gedächtnis in Djungarischen Hamstern untersucht, welche nach der Lernphase entweder einem Schlafentzug unterzogen wurden oder eine spontane Tagestorpor-Episode durchliefen. Der Schlafentzug wirkte sich negativ auf die Fähigkeit aus, bekannte von neuen Objekten zu unterscheiden (familiarity and recency memory) bzw. sich an komplexe räumliche Szenen zu erinnern (complex spatial scene retrieval). Tagestorpor-Episoden schränkten das Gedächtnis für komplexe räumliche Szenen ebenfalls ein, jedoch in einem geringeren Masse. Die Ergebnisse dieser Studie zeigen, dass Schlafmangel oder sensorische Stimulation

während des Schlafentzugs in einer Gedächtnisstörung resultieren können. Daraus folgt, dass der Schlaf bei Hamstern entweder aktiv an der Konsolidierung von Erinnerung an Objekte beteiligt ist, oder aber eine interferenzfreie Bedingung für die Gedächtnis-Konsolidierung schafft.

Die Bedeutung der zeitlichen Koordination des Schlafs während der Konsolidierungsphase wurde in Mäusen mittels eines Objekterkennungstests untersucht. Die Tiere wurden entweder sofort nach der Lernphase oder 6 Stunden später einem 6-stündigen Schlafentzug unterzogen. Bei der Unterscheidung zwischen einem neuen und zwei bekannten Objekten versagten ausschliesslich die Mäuse, bei denen der Schlafentzug direkt im Ausschluss an die Lernphase durchgeführt worden war. Im Gegensatz dazu zeigte der verzögerte Schlafentzug keinen negativen Einfluss auf die Gedächtnisleistung der Tiere. Somit beeinträchtigte der Schlafentzug oder die zusätzliche Aktivität der Tiere während des Schlafentzugs deren Fähigkeit, Objekte wieder zu erkennen, jedoch nur dann wenn der Schlafentzug sofort nach dem Lernen erfolgte.

Zusammengefasst zeigen diese Ergebnisse, dass Schlaf nach einer Tagestorpor-Episode homeostatisch reguliert ist, dass die Schlafregulation jahreszeitlich bedingte Aspekte aufweist und dass der Schlaf eine zentrale Rolle in der Gedächtniskonsolidierung bei Nagetieren spielt.

INTRODUCTION

1. Mammalian sleep

Sleep is a periodical state of quiescence that is characterized by reduced responsiveness to external stimuli and quick reversibility to wakefulness. Sensory information processing is minimal during sleep and no interaction with the environment occurs. Every species has its own elements of species-specific sleep behavior, which include sleep posture, choice of sleeping site and the timing of daily sleep. In contrast to humans exhibiting a monophasic sleep, most mammals have a polyphasic sleep pattern, with sleep episodes throughout the 24 h interrupted by periods of wakefulness (e.g. (Tobler, 1995)). In mammals, in addition to species-specific sleep behavior, additional criteria are used to define sleep, consisting in characteristic changes in the electroencephalogram (EEG) and electromyogram (EMG).

Sleep is a ubiquitous behavior, but its purpose is still unclear. Several functions have been proposed. They include energy conservation (Berger, 1975, Glotzbach and Heller, 1976, Walker and Berger, 1980), brain detoxification, tissue "restoration", thermoregulation (Heller, 2005), memory consolidation (Crick and Mitchison, 1983, Siegel, 2001, Siegel, 2005a) and brain plasticity associated with synaptic homeostasis (Tononi and Cirelli, 2006). The investigation of sleep and its regulation in rodents is likely to bring insights into its origins and functions and may contribute to the understanding of human sleep which would allow specific treatment of different sleep disorders.

1.1. Vigilance states

Three fundamental vigilance states: non-rapid eye movement (NREM) sleep also called slow wave sleep or delta sleep, paradoxical (Jouvet and Michel, 1959) or rapid eye movement (REM) sleep and wakefulness can be identified in rodents on the basis of the EEG and EMG. Sleep onset is associated with a slowing of the EEG activity, and a rising of the EEG amplitude, and a concomitant decrease in muscle tone, followed by the appearance of spindles and by slow waves (0.75-4.0 Hz) of relatively high amplitude. Spindle activity varies among species (e.g. 11 Hz in dogs, 8-11 Hz in the opossum, 10-16 Hz in rats, 10-13 Hz in mice and 12-16 Hz in primates) (Zepelin et al., 2005).

Sleep consists also of REM sleep (or paradoxical sleep; see also page 11), which is characterized by tonic and phasic events. Tonic events include desynchronized, low-amplitude EEG activity and postural atonia. Eye movements, twitching of the extremities, cardioresperatory irregularity and largely inhibited thermoregulatory responses belong to the phasic events. In animals, in addition to EEG and EMG, hippocampal theta activity and ponto-geniculo-occipital (PGO) spikes (for review (Datta, 1997) and (Sakai et al., 1976, Kaufman and Morrison, 1981)) can be recorded. Theta activity in rodents can be recorded by intracranial electrodes placed over parietal or occipital cortex and it is less evident in the frontal regions. A PGO spike is a field potential that can be recorded from the pons, the lateral geniculate nucleus and the occipital cortex. Paradoxically, although REM sleep was found to be the deepest state of sleep due to high thresholds of arousal and

sensory-motor reflexes, cortical activation during this sleep state is as high as during waking.

The alternation of NREM sleep with episodes of REM sleep represents the NREM-REM cycle, the basic unit of sleep. Duration of the sleep cycle as well as daily sleep quotas and percentage of REM sleep during 24 hours varies among species (for review (Zepelin et al., 2005)). For example in hamsters daily sleep quotas make up to 14 hours, while REM sleep occupies approximately 3 hours.

The third vigilance state, waking, is characterized by cortical activation with low-amplitude fast EEG activity and behavioral arousal that is evident in a high tone in postural muscles, particularly in the neck.

1.2. Neurophysiology of the vigilance states

NREM sleep. NREM sleep is characterized by three main oscillations at the EEG level: spindles (7-14 Hz), delta waves (1-4 Hz) and slow oscillations (~0.5-1 Hz). Spindle waves occur during the beginning of the NREM sleep. It has been shown that spindles can be generated after decortication in the thalamus, however, the corticothalamic volleys are important in triggering and synchronizing spindles throughout the thalamocortical systems (Steriade and Wyzinski, 1972, Contreras et al., 1996a, Contreras et al., 1997). Spindle waves are generated by the synaptic interactions between thalamic reticular (RE) and thalamocortical (TC) neurons (Steriade et al., 1993b, Bal et al., 1995). GABAergic RE neurons generate rhythmic inhibitory postsynaptic potentials (IPSPs) (Steriade et al., 1985, Bal et al., 1995) and act as pacemakers for spindles generation (Steriade et al., 1985, Steriade et al., 1987).

The cellular mechanisms responsible for the initiation of spindles in the RE nucleus are not completely understood, although they have been investigated intensively *in vitro* and *in vivo* intracellular studies. It has been hypothesized that hyperpolarization of the dendrites of RE neurons deinactivates a low-threshold Ca^{2+} current that triggers a Ca^{2+} spike. The spike is followed by hyperpolarization in dendrites of postsynaptic RE neurons and leads to initiation of spindle oscillation (Steriade et al., 1987). This idea was recently confirmed in the combined intracellular and extracellular recording *in vivo* of the cat RE nucleus. Some RE neurons (30%) fired spike bursts that were temporally related to the hyperpolarization in adjacent neurons (Fuentealba et al., 2004b). It has been suggested that such hyperpolarizations may lead to spindles and that they are generated locally in the RE nucleus. A recent *in vivo* study has shown that in addition to chemical synapses, electrical coupling is involved in the synchronization of spindle activity within the RE nucleus (Fuentealba et al., 2004a). It has been further shown in intracellular recording of RE neurons in cats under anesthesia that some RE neurons display membrane bistability (Fuentealba et al., 2005). Membrane bistability consists of two different states of membrane potentials, associated with different degree of neuronal responsiveness. An active membrane state, in contrast to a silent one, can modify the influence of depolarizing and hyperpolarizing cortical inputs. Bistable RE neurons (20%) fired tonically during spindles, whereas the remaining RE neurons fired rhythmic spike-bursts (Contreras et al., 1992). It has been suggested that bistable and non-bistable RE neurons could play a role in the induction of

different patterns of spindles in TC cells (Fuentealba et al., 2005). The functional role of spindles is considered to be the blockade of synaptic transmission through the thalamus (Steriade et al., 1969, Timofeev et al., 1996), leading to a disconnection of the cortex from sensory input.

It has been postulated that TC cells hyperpolarize progressively with the deepening of NREM sleep (Steriade, 2003). Thus, TC neurons display spindles at membrane potentials around -60 mV. When membrane potentials become more negative (-65 or -70 mV), spindles decrease in amplitude and delta waves appear.

The delta sleep oscillation (1-4 Hz) has two components. The mechanisms of generation of cortical delta waves are purely understood. It survives thalamectomy (Villablanca and Salinas-Zeballos, 1972, Steriade et al., 1993c, Villablanca, 2004). It has been shown *in vitro* that a small population of cortical neurons from layers IV and V (most of them pyramidal-shaped cells) respond to depolarizing current with a sequence of high threshold spikes (Connors et al., 1982). The frequency of these oscillations is 3-4 Hz. It has been hypothesized that cortical delta waves contribute to the polymorphism of the EEG (Amzica and Steriade, 1998). These oscillations represent the only delta activity that is induced by depolarization.

The other, clock-like component in the delta frequency band (1-4 Hz) is generated by individual thalamocortical neurons. It has been described *in vitro* in dorsal lateral geniculate neurons of the guinea pig and cat (McCormick and Pape, 1990, Leresche et al., 1991) and *in vivo* in motor, sensory, associational and intralaminar thalamic nuclei of the cat (Steriade et al., 1991, Dossi et al., 1992). It is present after decortication (Dossi et al., 1992). The basic mechanism of thalamic oscillation is based on two currents: the low-threshold transient Ca^{2+} current and the hyperpolarization-activated cation current (McCormick and Pape, 1990, Soltesz et al., 1991). The after-hyperpolarization of the Ca^{2+} current activates the cation current, which in turn triggers the Ca^{2+} current, promoting the rhythmicity of this oscillation.

Slow oscillations (<1 Hz; (Steriade et al., 1993d)) were observed in the cortex after thalamic lesions (Steriade et al., 1993c, Sanchez-Vives and McCormick, 2000), but were absent in the thalamus of decorticated cats (Timofeev and Steriade, 1996). These findings led to the conclusion that slow oscillations have cortical origin (Amzica and Steriade, 1995). Slow oscillations consist of a cyclic fluctuation of the neuronal membrane potential between two voltage levels: a depolarizing phase (“up state”) and a hyperpolarizing phase (“down state”), characterized by the absence of network activity (Steriade et al., 1993d, Wilson and Kawaguchi, 1996). The mechanism of the slow oscillation is not completely understood. It has been shown in naturally sleeping animals that cortical GABAergic interneurons do not fire during the hyperpolarizing phase and therefore, do not maintain it (Steriade et al., 2001, Timofeev et al., 2001). Recently, it has been shown in a cortical slice preparation that at a high concentration of extracellular K^+, cortical slices can oscillate in the frequency range of slow sleep oscillations (Sanchez-Vives and McCormick, 2000). Similar observations were obtained *in vivo* in a cortical slab preparation, when the gyrus of cortex was synaptically isolated from the rest of the brain, but a natural blood supply remained (Timofeev et al., 2000a). It has been suggested that the hyperpolarization-activated persistent Na^+

current is involved in the maintenance of the depolarizing states of the membrane potential during slow-wave sleep (Timofeev et al., 2000b).

Intracellular studies have shown that the hyperpolarization phase of the slow oscillation is associated with disfacilitation (a temporal absence of synaptic activity) leading to synaptic silence in intracortical and TC networks (Contreras et al., 1996b, Timofeev et al., 2001). Disfacilitation may be explained by a progressive depletion of extracellular calcium during the depolarizing phase of the slow oscillation (Massimini and Amzica, 2001). Thus, it has been suggested that the synchronous activation of cortical neurons during the depolarizing phase would lead to a progressive depletion of extracellular calcium due to a calcium entry at the postsynaptic sites. At the presynaptic sites, synaptic efficacy will progressively decrease due to the high sensibility of transmitter release to extracellular calcium concentration.

It has been proposed that summation of spontaneous miniature EPSPs during the hyperpolarizing phase of the slow oscillation activates the persistent sodium current and depolarizes the membrane of pyramidal neurons that triggers spikes and generates the next depolarizing phase (Timofeev et al., 2000a, Bazhenov et al., 2002). Spontaneous miniature synaptic activity is caused by action-potential-independent release of transmitter vesicles.

Long-lasting hyperpolarizations of cortical neurons are absent during REM sleep and waking (Steriade et al., 2001) because of an increased release of acetylcholine in the cerebral cortex (Celesia and Jasper, 1966, Steriade et al., 1993a).

Glial cells are also involved in generation of sleep oscillations. It had been shown not long ago by intracellular recording in naturally behaving cats that glial activity does not simply reflect neuronal activity (Amzica and Massimini, 2002). Thus, simultaneous recording from neurons and glia during a slow seep oscillation showed that two cells display coherent activities. Moreover, the onset of the depolarizing phase of the slow oscillation started in neurons and followed in glial cells. In contrast, the beginning of the hyperpolarized phase was initiated in glial cells and was followed by neurons. It has been hypothesized that synchronous sleep activity (i.e. slow sleep oscillation) is supported by the presence of functional glial cells (Amzica et al., 2002). Recently the confirmation of previous *in vitro* studies was obtained *in vivo*. Cholinergic activation of glial cells led to their hyperpolarization and was associated with neuronal depolarization (Seigneur et al., 2005). Therefore, cortical oscillations during NREM sleep result from the complex interactions between neurons and glia.

REM sleep and waking. In 1959, M. Jouvet and F. Michel discovered in cats a new sleep phase that they called paradoxical sleep (Jouvet and Michel, 1959). This sleep phase was characterized by a complete disappearance of muscle tone in association with a cortical activation and rapid eye movements. Follow-up studies revealed that the oral pontine reticular formation of the brainstem contains neurons which are crucial for the onset and maintenance of REM sleep (for review (Luppi et al., 2004, McCarley, 2004, Jones, 2005)). Specifically, cholinergic cells of the laterodorsal and pedunculopontine tegmental nuclei (LDTg and PPTg) promote REM sleep by initiating processes of forebrain activation and peripheral muscle atonia. In contrast to original

work, recent studies show that the locus coeruleus is not involved in the tonic EEG desynchronization and does not play a critical role in the initiation and maintenance of REM sleep (Sakai and Crochet, 2004).

Theta waves lead to the rhythm that is typical for REM sleep and waking in animals (Jouvet, 1969, Vanderwolf, 1969). The theta oscillation (3-12 Hz in rodents, (Bland and Colom, 1993)) is most regular in frequency and largest in amplitude in the stratum lacunosum-moleculare of the hippocampal CA1 region. It is present also in the dentate gyrus and the CA3 region. Field oscillations at theta frequencies have been observed also in several cortical structures, but none of these structures was capable of generating theta activity on its own (for review (Buzsaki, 2002)). The medial septum and diagonal band of Broca area (MS-DBB) are assumed to be the rhythm generators of theta waves (Petsche et al., 1962). However, it is still unclear whether they are independent pacemakers or whether the rhythmic firing of their neurons depends on hippocampal and entorhinal feedback as suggested by several computational models (Borisyuk and Hoppensteadt, 1999, Denham and Borisyuk, 2000, Wang, 2002).

Several decades ago the double dipole model (Holsheimer et al., 1982) and the model of gradual phase shift (Leung, 1984) were developed to explain the mechanism of theta rhythm generation. Later, two types of septal projection cells, i.e. cholinergic and GABAergic, were discovered (Stewart and Fox, 1989). It has been hypothesized that the rhythmic cholinergic projections of septal cells end on both pyramidal cells and interneurons and provide slow depolarization of both cell types. GABAergic projections rhythmically hyperpolarize the hippocampal interneurons. It has been suggested that the phase of firing for a hippocampal interneuron is set by firing phases of its excitatory and inhibitory septal inputs (Stewart and Fox, 1990). However, complete destruction of the septohippocampal cholinergic projections resulted in survival of some theta activity in the hippocampus (Lee et al., 1994). Therefore, the integrity of the GABAergic projection was sufficient to partially maintain hippocampal theta activity. It has been hypothesized that cholinergic neurons serve to increase the population phase-locking of septal cells and to regulate the magnitude of hippocampal theta (Lee et al., 1994). Moreover, the frequency of hippocampal theta might be determined by the polarization level of GABAergic neurons, while the amplitude of theta might be determined by the number of oscillating cells in the septum and by their synchrony (Lee et al., 1994). These suggestions were confirmed in extracellular recordings in behaving and anesthetized rats (King et al., 1998, Brazhnik and Fox, 1999).

1.3. Sleep regulation

A fundamental characteristic of sleep is its homeostatic regulation. Three distinct processes: a homeostatic, a circadian and an ultradian process, underlie sleep regulation. Sleep homeostasis is represented by “process S”, which rises during waking and declines during sleep. The circadian process is independent of prior sleep-wake history, and modulates the timing and propensity of sleep. The two-process model of sleep regulation postulates that the timing and structure of sleep are determined by the interaction of the homeostatic and the circadian process (Borbély, 1982).

Many experiments led to the formulation of the model and after the model was formulated it stimulated many experiments. Sleep deficits

invariably elicit compensatory increases in the duration and intensity of sleep. SWA (0.75-4.0 Hz) in NREM sleep is considered to reflect its intensity and is used as an electrophysiological correlate of the homeostatic process S (Achermann and Borbély, 2003, Tobler, 2005). SWA changes as a function of prior sleep and wake history. Thus, the initial increase in SWA after spontaneous or induced wakefulness (sleep deprivation) and its monotonic decline during recovery sleep were shown in humans (Borbély et al., 1981), several mouse strains (Tobler et al., 1997, Huber et al., 2000a), rats (Borbély et al., 1984, Trachsel et al., 1986, Franken et al., 1991, Schwierin et al., 1999), hamsters (Tobler and Jaggi, 1987, Deboer et al., 1994), ground squirrels (Larkin and Heller, 1998, Strijkstra and Daan, 1998), guinea pigs (Tobler and Franken, 1993), cats (Tobler and Scherschlicht, 1990) and rabbits (Tobler et al., 1990). EEG power density in the range of sleep spindles (7-14 Hz) shows in part an inverse relationship to SWA (e.g. (Trachsel et al., 1988, Aeschbach et al., 1997, Vyazovskiy et al., 2004a)). It has been shown recently that in the waking EEG, theta activity (e.g. 5-8 Hz in humans, 5-7 Hz in rats) (Finelli et al., 2000, Vyazovskiy and Tobler, 2005) may serve as a marker of process S. The rise rate of theta activity during prolonged waking is correlated with the increase of SWA during recovery sleep (e.g. (Finelli et al., 2000, Vyazovskiy and Tobler, 2005)). In contrast to SWA, theta activity exhibits a marked circadian modulation (Welsh et al., 1985). Therefore, a common homeostatic process may be evident in the EEG in both waking and sleep.

The ultradian process occurs within sleep and is represented by the alternation of NREM sleep and REM sleep.

REM sleep is also homeostatically regulated. Benington and Heller (Benington and Heller, 1994) proposed that REM sleep pressure increases exclusively during NREM sleep. According to their hypothesis the daily amount of REM sleep would be regulated through NREM sleep only. However, data obtained in rats, which were subjected to REM sleep deprivation, indicated that a rise in REM sleep propensity occurs whenever this sleep state is prevented, i.e. during both NREM sleep and waking (Endo et al., 1997a). More recently Franken (Franken, 2002) postulated that REM sleep is regulated by two processes. The “long-term” homeostatic process determines the daily amount of REM sleep and is NREM sleep independent, whereas the “short-term” process determines the NREM-REM sleep cycles. This implies that REM sleep propensity accumulates during both wakefulness and NREM sleep.

In contrast to NREM sleep, there is no obvious EEG marker of REM sleep intensity. Therefore, it is difficult to model REM sleep regulation. Many studies showed that a rise in “REM sleep pressure” is manifested only in an increased duration of REM sleep (humans (Beersma et al., 1990, Endo et al., 1998), rats (Endo et al., 1997a)). However, data obtained in humans showed that alpha activity may represent a correlate of REM sleep propensity in the sleep EEG (Endo et al., 1998, Roth et al., 1999).

1.4. Regional aspects of sleep

Sleep not only represents a global brain phenomenon encompassing the entire brain, but can also be important for local recovery functions. It has been proposed that slow waves in NREM sleep may reflect local processes associated with the stimulation of synapses that were insufficiently used

during preceding wakefulness to maintain neuronal connections (Krueger and Obál, 1993) or with synaptic downscaling following synaptic potentiation that occurred during waking (Tononi and Cirelli, 2003, Tononi and Cirelli, 2006). An alternative hypothesis suggested that brain glycogen levels, depleted due to brain activity during wakefulness, may be restored during sleep (Benington and Heller, 1995).

Experimental data indicate that sleep does not necessarily involve the entire brain. Spectacular examples are marine animals (i.e bottlenose dolphins, sea lions) which exhibit deep slow-wave sleep only in one hemisphere, while the other hemisphere is awake (Mukhametov et al., 1977, Lyamin et al., 2002). When in dolphins one brain hemisphere (right or left) was deprived of slow-wave sleep, only that hemisphere showed a rebound of slow-wave sleep (Oleksenko et al., 1992), suggesting that sleep pressure can increase during SD in a part of the brain (i.e. in the deprived hemisphere only). In contrast to dolphins, birds spend only 12-30% of total sleep time in unilateral sleep and in the remaining time show SWA in both hemispheres (Rattenborg et al., 1999). In contrast, in dolphins SWS never occurs simultaneously in both hemispheres. Behaviorally the interhemispheric asymmetry is often associated with unilateral eye closure.

Brain asymmetries have been observed in several other species. In humans, unilateral activation of the left somatosensory cortex by vibration of the right hand during wakefulness resulted in a shift of the EEG power in sleep towards the hemisphere contralateral to the stimulated hand (Kattler et al., 1994). In another study in humans, local SWA changes in NREM sleep were induced by a learning task involving specific brain regions (Huber et al., 2004). A beautiful example of use-dependent changes in sleep in animals is the vibrissae stimulation experiment. An unilateral sensory stimulation of rat vibrissae during 6 h waking led to an interhemispheric shift of EEG power in the 0.75-6.0 Hz range in NREM sleep towards the cortex contralateral to the stimulated whiskers (Vyazovskiy et al., 2000). This finding was supported by the regional pattern of brain metabolic activation in rats (Vyazovskiy et al., 2004b). Thus, SD combined with unilateral whisker stimulation in an enriched environment induced an asymmetry in 2-DG uptake in the barrel cortex. Higher 2-DG uptake values were found in the hemisphere contralateral to the whisker stimulation. The regional pattern of metabolic activity after SD was similar to EEG topography during sleep. It has been suggested that the increase of SWA in NREM sleep depends on previous activation of cortical areas (Vyazovskiy et al., 2004b). These data support the notion that sleep regulation has a local, use-dependent component.

In addition to the interhemispheric asymmetry of the sleep EEG, topographic differences along the antero-posterior axis were described in several species. These differences had state-dependent and frequency-dependent character. In humans, EEG power in the low frequency band in NREM sleep was higher in the frontal derivation than in the parietal derivation (Werth et al., 1996, Werth et al., 1997a). These regional differences were enhanced after 40-h SD (Cajochen et al., 1999, Finelli et al., 2001). Similar regional differences were found also in rodents under baseline conditions (rats, (Schwierin et al., 1999)). Figure 1 illustrates state- and frequency-specific topographic differences in EEG activity during an undisturbed baseline in the Djungarian hamster. A further increase of frontal power in

NREM sleep compared to occipital power was observed in rats in the 2-4 Hz band after 24 h SD (Schwierin et al., 1999) and in two mouse strains in the 0.75-2.5 Hz band after a 6 h SD (Huber et al., 2000b). Taken together, these data led to the hypothesis that there may be regional differences in the dynamics of the homeostatic component of sleep regulation and that sleep has local, use-dependent features.

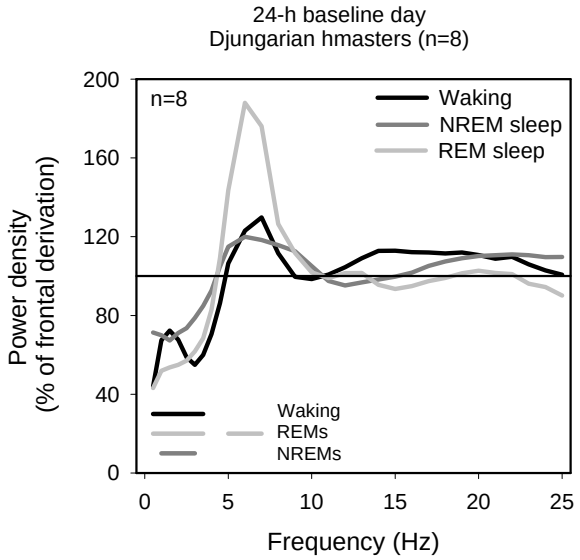

Figure 1. Frontal predominance in the EEG power density during a 24-h baseline day in the Djungarian hamster (n=8).
Power density of the parietal derivation expressed as percentage of frontal derivation (100 %; mean ± SE) during Waking, NREM sleep and REM sleep. Lines in the bottom indicate significant differences between the two derivations during the three vigilance states ($p<0.05$, paired *t*-test).

It has been shown that also spindle activity exhibits topographic changes (Werth et al., 1996, Werth et al., 1997b, Schwierin et al., 1999). Prolonged waking induced a marked decrease of power in the spindle range with the largest reduction over the frontal regions both in humans (Finelli et al., 2001) and in rats (Schwierin et al., 1999). The regional differences in sleep EEG spectra indicate that sleep is not only a global phenomenon, but reflect local brain processes with different regional involvement of neuronal populations.

In the first paper of the present thesis (Palchykova et al., 2002) regional EEG differences were investigated in Djungarian hamsters which were subjected to SD or spontaneously underwent episodes of daily torpor.

2. Regulation of sleep in the Djungarian hamster

2.1. Effects of seasonality and photoperiod on sleep

Long-term timing mechanisms that allow organisms to anticipate unfavorable environmental events and to optimize survival and reproductive success are widespread in nature. Photoperiodism and circannual rhythm seem to be used by mammals for long-term timekeeping (for review (Lincoln et al., 2003)). Photoperiodism registers the change of day length in the annual cycle and translates this information into timed control of physiology and behavior (Tamarkin et al., 1985). The physiological adaptations induced by the shortening of the photoperiod are manifold and include suppression of prolactin production, reduction in food intake and body weight, changes in pelage color and density, gonadal regression and suppression of breeding, and in some species hibernation or daily torpor. Circannual rhythm is a feature in many long-living mammals that survive and breed over several seasons.

These animals express annual cycles in nature, and continue to express circannual cyclicity indoors under constant conditions for many years (Gwinner, 1989, Woodfill et al., 1994, Gwinner, 2003). In some animals (e.g. hibernating ground squirrels, tropical fruit bats) the intrinsic circannual cycle predominates irrespectively of the photoperiod, while in others (e.g. sika deer, Suffolk sheep) both circannual timing and photoperiod are combined to regulate seasonality (Lincoln et al., 2003).

Photoperiod is interpreted by the master clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus (Schwartz et al., 2001). Photoperiodic information reaches the SCN via the retinohypothalamic tract. Multiple circadian clock genes, which are believed to form the molecular basis of circadian clocks, are expressed in the SCN in a photoperiod-dependent manner (Lincoln et al., 2003, Sumova et al., 2004, Johnston, 2005). The key neuroendocrine output of the clock is the rhythmic secretion of the pineal hormone, melatonin (Arendt and Skene, 2005). Melatonin is secreted at night, while day-length information is encoded in the duration of its nocturnal secretion. The melatonin signal is “decoded” in melatonin-sensitive tissues and drives physiological changes in the organism. It has been shown in the ovine and in the Syrian hamster that the pars tuberalis of the pituitary gland is the main melatonin target tissue. It secretes a prolactin-releasing factor tuberalin (Hazlerigg et al., 1996, Stirland et al., 2001), which is involved in the seasonal prolactin secretion and regulation of the reproductive system (Hazlerigg et al., 1993, Morgan et al., 1996).

It is not clear whether seasonality is a direct response of the body to environmental factors such as ambient temperature and photoperiod or whether it is based on an annual rhythm regulated by an endogenous mechanism (Kohsaka et al., 1992). Many rodents do not exhibit repeated annual cycles when they are kept in seasonally constant conditions. For example, Djungarian hamsters exposed to a chronic long photoperiod remain in the summer state continuously. Exposure to a short photoperiod or decreasing the day length in late summer initiates a transition to the winter phenotype. However, after approximately 20 weeks, despite continuous maintenance at short photoperiod, Djungarian hamsters spontaneously revert to summer physiology (Hoffmann, 1973). Thus, blood prolactin concentrations and pelage pigmentation have also been shown to return to summer values within 38 weeks (Figala et al., 1973, Bockers et al., 1997, Kuhlmann et al., 2003). This phenomenon is known as a loss of responsiveness, or refractoriness, to prolonged short photoperiod. Refractory responses allow the animal to prepare towards its spring physiology. Once refractoriness is established, exposure to a long photoperiod is required to break refractoriness and return to the photosensitive state, as was shown for golden hamsters (Stetson et al., 1977). Therefore, refractoriness represents a critical feature in the biology of seasonal mammals.

Refractoriness is thought to occur at melatonin target sites (Bittman, 1978, Freeman and Zucker, 2001), but the cellular and molecular mechanisms underlying this process are poorly understood. Interestingly, several studies in Syrian and Djungarian hamsters showed that the melatonin rhythm was not altered by entering refractoriness, but matched the ambient day length (Bockers et al., 1997, Johnston et al., 2003). However, melatonin receptor density in the pars tuberalis of photorefractory Syrian hamsters kept

under a short photoperiod reached the level typical for animals fully adapted to the long photoperiod. It has further shown in Djungarian and Syrian hamsters that endogenous secretion from the pars tuberalis spontaneously switched from the short to the long photoperiod. This resulted in elevation of prolactin synthesis despite a persistent short photoperiod-like melatonin profile and despite a low expression amplitude of the clock genes *per1* and of the inducible cAMP early repressor (ICER) mRNA (Bockers et al., 1997, Johnston et al., 2003). Hence, in refractory animals endocrine output from the pars tuberalis varies with changes in photoperiod sensitivity. In contrast, clock gene expression continues to reflect the melatonin signal corresponding to the short photoperiod. It has been hypothesized that hypothalamic factors regulate prolactin homeostasis, whereas tuberalin drives the seasonal prolactin secretion (Lincoln and Clarke, 2002). Thus, although the seasonal cycle of mammals is initiated by external cues, there remains an endogenous aspect of its control.

A limited number of studies has addressed seasonal changes in sleep. In humans it has been shown that under winter short photoperiod conditions sleep duration is increased (Wirz-Justice et al., 1984, Wehr, 1991, Wehr et al., 1993) and timing of sleep is changed (Kohsaka et al., 1992). Only few studies in animals recorded the same individuals during summer and winter. A behavioral study in elephants in captivity found an increase in sleep duration of approximately 14 % during the winter months (Tobler, 1992). A large amount of sleep in winter and less sleep in summer was reported in 4 golden mantled ground squirrels and 4 captive prosimians, *Microcebus murinus* (Walker et al., 1980, Barre and Petterrousseaux, 1988). In laboratory studies artificial changes in photoperiod in rodents (rat, Siberian chipmunk, and Djungarian hamster) did not affect the amount of sleep, but induced a marked redistribution of sleep across 24 h (Borbély and Neuhaus, 1978, Dijk and Daan, 1989, Franken et al., 1995, Deboer and Tobler, 1996b, Deboer and Tobler, 1997). Moreover, a short photoperiod and low ambient temperature abolished the daily changes in SWA in NREM sleep in the Djungarian hamster (Deboer and Tobler, 1997). It is unknown whether the sleep-wake pattern in refractory animals corresponds to the short photoperiod or is changed to the long one. In the second paper (Palchykova et al., 2003) of this thesis the distribution of sleep and EEG power density within the SWA range were investigated in refractory Djungarian hamsters kept under constant short photoperiod and low ambient temperature.

2.2. Sleep after daily torpor and sleep deprivation

Special manifestations of sleep-like behavior such as daily torpor and hibernation do not seem to fulfill the functions of physiological sleep, since they are followed by a compensatory increase in sleep intensity. Hibernation is a hypometabolic state during which major physiological changes occur in the organism, including a remarkable decrease in body temperature and metabolism (Heldmaier et al., 1989), suspension of transcription and translation in the liver and concomitant low electrical brain activity (Snapp and Heller, 1981, Berriel Diaz et al., 2004). In contrast to hibernation, daily torpor, which also belongs to the hypothermic states, lasts in average 11 h (Geiser and Ruf, 1995), and the decrease of body temperature (T_{body}) to approximately 13 - 27 °C (Heldmaier and Ruf, 1992) is not as dramatic as the

temperature decrease observed in hibernators (Fig. 2). The amplitude of brain electrical activity decreases during daily torpor, but some EEG activity resembling NREM sleep is still present even at the lowest T_{body} (Fig. 3), while REM sleep cannot be identified below 25 °C.

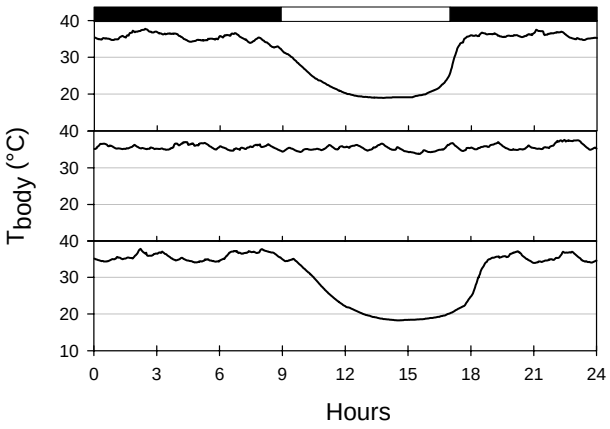

Figure 2. A representative 96-h continuous record of body temperature of a Djungarian hamster on a baseline day (middle panel) and the preceding and subsequent day each with an episode of daily torpor (upper and lower panel, respectively). Each data point represents a 2-min average value. The light-dark cycle (LD 8:16) is indicated by the white (light period) and black (dark period) bar on the top.

The entrance into hibernation and daily torpor is accomplished via NREM sleep (Walker et al., 1979, Deboer and Tobler, 1994). Animals retire to the nest, assume a characteristic heat-conserving curled-up posture, remain behaviorally quiescent, and exhibit elevated arousal thresholds. It has been suggested that daily torpor and hibernation evolved as an extension of NREM sleep, because it is well known that also during NREM sleep T_{body} is lower and is regulated at a lower level compared to quiet waking. On the basis of these findings, a common energy conserving function was proposed for hibernation, torpor and sleep (Walker et al., 1979, Berger, 1984, Heller, 1988).

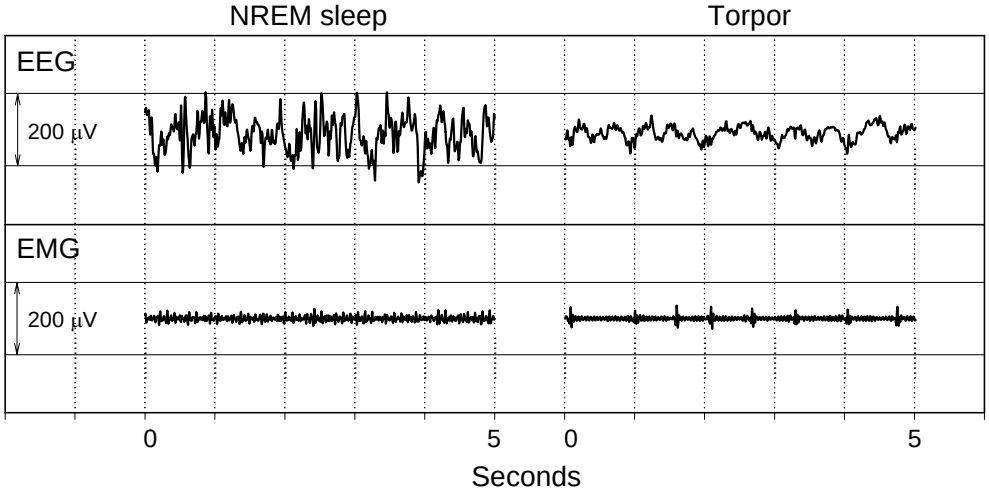

Figure 3. 5-s trace of raw EEG (μV) and EMG (μV) during NREM sleep and during an episode of daily torpor (T_{body} = 18.7 ºC) in the Djungarian hamster.

The torpid state cannot persist indefinitely during the hibernation season and is frequently interrupted by euthermic bouts of approximately 1 day. The mechanisms regulating these periodic arousals into euthermia are still unknown, but seem to be endogenous. Energetic costs of the recurring

arousals constitute 64 - 90% of the total energy expenditure during the hibernation season (for review (Geiser, 2004)). On average, entering torpor for a few hours saves 20% of energy compared to the amount required to remain euthermic for the same time period in Djungarian hamsters (Ruf and Heldmaier, 1992). However, even such a minor conservation of energy may represent an important energetic aid for survival during unfavorable environmental conditions.

The Djungarian hamster is a photoperiodic species, which engages regularly in daily torpor during the winter months. Specific adaptations, including gonadal regression, reduction in body weight, change in pelage color and redistribution of the daily sleep-wake activity precede the regular occurrence of daily torpor (Fig. 4). Entry into torpor generally occurs at the same time of day within the normal daily rest phase.

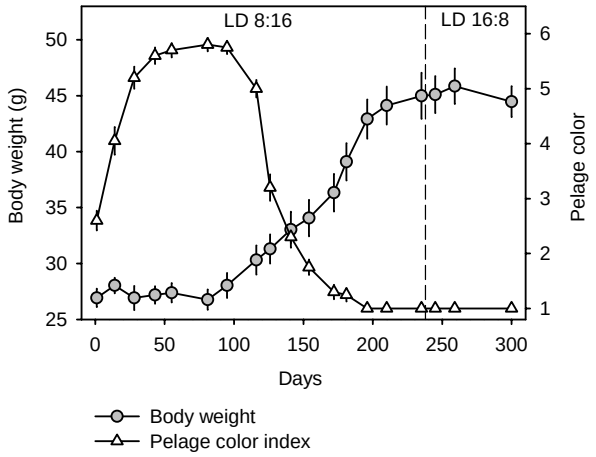

Figure 4. Changes in body weight (g) and a pelage color index (1-6; 1-summer pelage, 6 - winter pelage) in Djungarian hamsters (n=19) kept under short photoperiod (LD 8:16) and later under long photoperiod (LD 16:8).

Daily torpor or hibernation, expressed spontaneously in winter, may also occur at other times of year depending on food or water availability in insects, fishes, reptiles, 11 out of 26 orders of birds, 6 orders of mammals, including some primates (e.g. (Tsukada et al., 1993, Grigg et al., 2004)). It has been shown recently that a torpor episode can be induced artificially in some mammalian species by exposure to hydrogen sulfate (Struve et al., 2001, Blackstone et al., 2005) or by injecting 5'-adenosinemonophosphat (5'-AMP) (Zhang et al., 2006).

In contrast to the assumption that hibernation and torpor are an intense form of sleep, it was shown for ground squirrels (*Spermophilus parryii* and *Spermophilus lateralis*) and hamsters (*Phodopus sungorus*) that animals warming up from hibernation or daily torpor are predominantly asleep during the arousal process, and spend at least 60% of the euthermic period between hibernation bouts, in sleep. Spectral analysis of the EEG of ground squirrels and Djungarian hamsters revealed that the initial phase of euthermia is characterized by high levels of EEG power in NREM sleep in the SWA range (0.75 - 4.0 Hz). Moreover, SWA is high at the beginning of the euthermic interval and declines monotonically (Daan et al., 1991, Trachsel et al., 1991, Deboer and Tobler, 1994) with dynamics similar to the well-known changes in SWA after prolonged waking observed in humans (Borbély et al., 1981), rats (Borbély et al., 1984, Trachsel et al., 1986, Franken et al., 1991, Schwierin et al., 1999) and mice (Tobler et al., 1997, Huber et al., 2000b). This similarity

led to the postulation that animals incur a SD during hibernation and daily torpor (Daan et al., 1991, Trachsel et al., 1991, Deboer and Tobler, 1994).

A relationship between the changes in SWA in NREM sleep and the duration of prior waking has been established in numerous mammals (Tobler, 2005). Similarly, a positive correlation between duration of torpor episodes and SWA increase during subsequent sleep was found in hamsters and ground squirrels (Trachsel et al., 1991, Deboer and Tobler, 1996a, Strijkstra and Daan, 1997, Larkin and Heller, 1998, Deboer and Tobler, 2000). Moreover, the correlation was characterized by a saturating exponential curve (Deboer and Tobler, 1996a), implying that the kinetics of the build-up of sleep pressure during daily torpor and waking is similar. The detailed analysis of the dynamics of the process underlying the build-up of sleep pressure during episodes of waking and torpor in hamsters performed by Deboer and Tobler (Deboer and Tobler, 2003) revealed that the build-up of sleep pressure during torpor appeared to be slowed down by a factor of 2.75 by the lower brain temperature. This result provided additional arguments leading to the conclusion that processes occurring during daily torpor are similar to those occurring during SD. Thus, e.g. when hamsters were sleep deprived for 1.5 h immediately after emerging from torpor, an additional SWA increase above the level reached after SD alone occurred during the delayed recovery (Deboer and Tobler, 2000). A similar experiment in ground squirrels did not result in an additional increase in SWA (Larkin and Heller, 1998, Strijkstra and Daan, 1998). However, squirrels slept 13-20% of the SD time. This partial SD allowed considerable sleep to occur and might be responsible for the differences observed between these two species. Further studies are needed to clarify whether the magnitude of the temperature decrease or species differences play a role.

It has been shown in humans and in rats that selective attenuation or prevention of NREM sleep with high amplitude slow waves results in a SWA rebound in the recovery period (Dijk et al., 1987, Endo et al., 1997b). To test whether post torpor SWA is homeostatically regulated, which would be an indication of recovery from the sleep deficit incurred during torpor, hamsters were subjected to partial NREM sleep deprivation immediately after the termination of the episode of daily torpor. Such partial deprivation should result in an attenuation of the SWA rebound until recovery sleep would be allowed. Indeed, an increase of SWA was found after the termination of 1.5 h partial NREM sleep deprivation following torpor in the study described in the first paper of this thesis (Palchykova et al., 2002), leading to the conclusion that post-torpor SWA is homeostatically regulated.

3. Sleep and memory

3.1 Sleep stages and memory categories

The relationship between sleep and memory has become one of the most popular topics in neuroscience (for review (Stickgold, 2005, Walker, 2005)). Sleep consists of two distinct states, NREM sleep and REM sleep, that differ in the level of brain activation and responsiveness to external stimuli. On the other hand, it is believed that there is a broad spectrum of memory categories in the brain. Moreover, the processes which create and sustain memory appear to be equally diverse. In humans, the most widely used classification

of memories distinguishes between declarative and non-declarative memory, while for animal memories such a clear classification does not exist. Declarative memory involves conscious recollection of fact-based information and comprises memories for people, places, objects and events. It can be subdivided into two main categories: episodic and semantic memory. Declarative memory involves a special anatomical system in the medial temporal lobe structures and the hippocampus (Graham and Gaffan, 2005). Non-declarative memory is the memory for perceptual and motor skills, actions and habits. It is expressed without conscious recall of past experience and appears to be less dependent of the medial temporal lobe structures. However, in real life memories are usually composed of both declarative and non-declarative components.

Memory creation and memory development are also not simple events. It has been proposed that initial encoding (acquisition) of memory is followed by several distinct stages, including memory consolidation, integration with previous experiences and knowledge, translocation and loss or active erasure of memory. It is believed that after retrieval or recall the memory representation becomes unstable and requires a period of reconsolidation (for review (Dudai and Eisenberg, 2004)). Memory consolidation is the most studied stage of memory development. During consolidation, a memory trace becomes resistant to interference from competing or disrupting factors in the absence of further practice (McGaugh, 2000). Memory consolidation is subdivided into two phases: a stabilization phase that seems to occur mainly during waking and an enhancement phase that depends on sleep (Walker, 2005). It is suggested that long-term memories are initially registered in both the hippocampal formation and the relevant area of the neocortex and then shifted from the hippocampus to the neocortex. After that the neocortex can independently maintain a specific internal representation of the memory and actualize it at retrieval (Dudai, 2004).

Support for the hypothesis that sleep contributes to memory development comes from both animal and human studies, but the mechanisms of sleep-related facilitation of memory are poorly understood. It has been suggested that sleep might either provide optimal conditions of non-interference for memory consolidation or, alternatively, might actively facilitate consolidation. There are two main theories postulating a facilitatory role of sleep in the consolidation of memories. The dual-process hypothesis suggests a functional dichotomy in humans: REM sleep would serve for consolidation of procedural memories, whereas NREM sleep would facilitate consolidation of declarative memories (Karni et al., 1994, Smith, 1995). The two-step process hypothesis rather proposes a complementary role of NREM and REM sleep for the consolidation of both declarative and procedural memories (Giuditta et al., 1985, Giuditta et al., 1995, Stickgold, 1998). Memory recall in form of neuronal reverberation is suggested to occur mainly during NREM sleep, whereas memory storage associated with the expression of plasticity related genes seems to take place during REM sleep (Ribeiro and Nicolelis, 2004).

3.2 Changes in sleep following learning

It has been shown in rodents that not only the duration of prior wakefulness, but also waking experience has an impact on the structure of subsequent sleep. Thus, e.g. rearing in an enriched environment resulted in a significant

increase in the number and the duration of REM sleep episodes in CF1 mice and an enhanced recall in a brightness discrimination task (Gutwein and Fishbein, 1980). A number of studies in several inbred and outbred mouse and rat strains showed an increase in REM sleep following avoidance conditioning (Fishbein et al., 1974, Hennevin and Hars, 1987, Ambrosini et al., 1992) or a two-way shuttle avoidance task (Smith et al., 1980, Smith and Lapp, 1986). In addition, the amount of REM sleep and learning ability in an active avoidance task correlated positively in 7 inbred strains of mice (Pagel et al., 1973). In a recent study, an increase of pontine wave (P-wave) density in REM sleep was shown to follow conditioned avoidance learning and to correlate positively with task retention (Datta, 2000). Moreover, activation of the pons with carbachol prevented memory-impairing effects of a post-acquisition REM sleep deprivation (Datta et al., 2004). However, all these studies used the fear paradigm, and therefore, did not distinguish between the effects of learning and stress.

A number of studies recorded neuronal activity in freely moving rodents, songbirds, non-human primates and humans (Pavlides and Winson, 1989, Dave and Margoliash, 2000, Maquet et al., 2000, Hoffman and McNaughton, 2002). Correlation analysis showed that firing patterns of cells active during waking (i.e. during experience of novelty, spatial tasks) tended to recur during sleep (e.g., (Wilson and McNaughton, 1994, Dave and Margoliash, 2000, Ribeiro et al., 2004)). It has been postulated that hippocampal sharp waves may provide a mechanism for such neuronal reactivation and therefore for neuronal plasticity (Nadasdy et al., 1999). However, the neocortical reactivation observed in these studies was extremely subtle and decayed rapidly. The investigation of the cell firing patterns was restricted to the hippocampocortical loop, thus, questioning the global role of experience-dependent changes. Moreover, the brain reactivation was observed in highly trained animals, raising skepticism about the relevance of such events for the acquisition and for the consolidation of novel information. And last, reactivation has been reported to occur in all behavioral states, including waking, suggesting that this process is not specifically during sleep.

3.3 Effects of sleep deprivation on learning (acquisition) and memory consolidation

A different approach to investigate the role of sleep in memory is to deprive humans or rodents of sleep. If sleep is important for memory processing, then the lack of sleep is expected to induce learning and memory impairments. Two main strategies have been used to investigate the effects of SD on learning and memory: pre-acquisition and post-acquisition SD.

It has been shown in mice and rats that SD performed before the acquisition of several tasks, including the Morris water maze task (Beaulieu and Godbout, 2000, Guan et al., 2004), one- and two-way active avoidance learning (Stern, 1971, Gruart-Masso et al., 1995), passive avoidance (Linden et al., 1975), taste aversion (Danguir and Nicolaidis, 1976, Venkatakrishna-Bhatt et al., 1978) and fear conditioning (McDermott et al., 2003, Ruskin et al., 2004), affected learning in different ways. Neutral and positive memory events were most severely impaired by SD. In contrast, encoding of negative stimuli,

that are usually amygdala dependent, was more resistant to disruption by SD (Walker and Stickgold, 2005).

The effects of post-acquisition SD have been found to depend on the memory task, duration and timing of SD and on the species under investigation. Sleep deprivation-induced memory impairment has been observed in mice and rats in several behavioral tasks, including the Morris water maze task (Smith and Rose, 1996, Youngblood et al., 1999), the eight-arm radial maze task (Smith et al., 1998), active, passive and inhibitory avoidance (Shiromani et al., 1979, Kitahama et al., 1981, Marti-Nicolovius et al., 1988, Smith and Kelly, 1988, Wang et al., 2003, Datta et al., 2004, Silva et al., 2004), fear conditioning (Graves et al., 2003, Silvestri, 2005), fear potentiated startle (Su et al., 2004) and complex associative learning (Bjorness et al., 2005). In general, tasks with higher memory load were more sensitive to the disrupting effects of SD. However, animal sleep deprivation studies are often limited by the lack of appropriate controls, unspecific effects of SD, stress associated with the SD procedure, fatigue, sleepiness, reduced motivation and the lack of selectivity of REM sleep deprivation procedures, which are often accompanied also by a substantial loss of NREM sleep (Vertes, 2004, Siegel, 2005b, Vertes, 2005).

3.4 Sleep and brain plasticity

It is generally believed that memory formation depends on brain plasticity (for review (Lynch, 2004)). In the course of learning (acquisition) a new experience triggers a molecular cascade that induces synaptic and cellular alterations, posttranslational modifications, a modulation of gene expression and morphological synaptic remodeling. Long-term potentiation (LTP) is believed to be a basic mechanism that underlies memory formation (Kandel, 2001).

Several animal studies have investigated potential cellular and molecular mechanisms of brain plasticity and their relation to sleep. At the cellular level, 24 and 72 h REM sleep deprivation reduced the excitability of hippocampal neurons and impaired formation and maintenance of LTP within these neurons *in vitro* (Davis et al., 2003, McDermott et al., 2003). However, the properties of neurons in a slice preparation can be quite different from the *in vivo* situation because of lost connections between neurons. Albeit, recent *in vivo* studies also showed that 9 h REM sleep deprivation disrupt LTP induction in dentate granule cells of rats (Marks and Wayner, 2005). It was further shown that 5 days REM sleep deprivation as well as 4 h total SD impair LTP maintenance in rat hippocampal neurons (Romcy-Pereira and Pavlides, 2004, Kim et al., 2005a), but facilitate LTP in the medial prefrontal cortex (Romcy-Pereira and Pavlides, 2004). In summary, the effects of SD on brain plasticity appear to be region specific.

At the molecular level, it was demonstrated that the protein level of the nerve growth factor (NGF) in the hippocampus and of the brain-derived neurotrophic factor (BDNF) in the brainstem and cerebellum were decreased after 6 h of REM sleep deprivation in Wistar rats (Sei et al., 2000). In another study, the level of phosphorylated extracellular signal-regulated kinase (ERK) was shown to be reduced in the hippocampus after a Morris water maze task followed by total SD (Guan et al., 2004). Thus, molecular mechanisms involved in brain plasticity seem to be sleep-dependent.

It was shown that gene expression is differently regulated during sleep and wakefulness in rats (Tononi and Cirelli, 2001, Cirelli, 2002, Cirelli et al., 2004, Terao et al., 2005) and in Drosophila (Cirelli et al., 2005). In particular, approximately 100 genes were found to be specifically up-regulated during sleep (Cirelli et al., 2004). While the majority of plasticity related genes were down-regulated during sleep (Cirelli and Tononi, 1998), a specific up-regulation of zif-268 during REM sleep occurred in rats after exposure to an enriched environment (Ribeiro et al., 1999). Moreover, the expression of zif-268 propagated gradually from the hippocampus to extrahippocampal regions as REM sleep recurred, suggesting a progressive activation of the hippocampal-neocortical network during REM sleep (Ribeiro et al., 2002). Thus, it is possible that a selective up-regulation of plasticity-related genes may occur during sleep in circuits which undergo synaptic remodeling.

Nothing is known yet about the relationship between recognition memory and sleep in rodents. In the last two papers the importance of sleep and its timing on recognition memory consolidation was investigated. The effects of a sleep loss associated with either SD or daily torpor on memory were tackled in two rodent species, Djungarian hamsters and mice. Daily torpor is associated with a complete absence or low sensory stimulation and can provide a model of SD without interference. Therefore, the question whether sleep actively facilitates memory consolidation or provides optimal conditions of non-interference was addressed in torpor animals.

3.5 Memory consolidation and stress

There is literature demonstrating that some aspects of cognitive functioning might be sensitive to stress both in humans and animals. Hormones of the hypothalamus-pituitary-adrenal (HPA) axis, glucocorticoids, secreted during stressful experience lead to either enhancement or impairment of memory depending on the intensity and duration of stress, the type of memory system that is tested, strain, gender and age of animals (e.g. (Garcia et al., 2000, Pothion et al., 2004, Bowman, 2005)). It has been repeatedly shown that glucocorticoids have multiple effects in the brain, including modulation of glucose transport, neuronal excitability, synaptic plasticity, synaptic spine density and neurogenesis (for review (Wolf, 2003)). In rats and mice stress or high concentrations of circulating corticosterone inhibited LTP induction *in vitro* and *in vivo* (for review (Lynch, 2004) and (Hui et al., 2005, Kim et al., 2005b, Kavushansky et al., 2006)). Behavioral experiments revealed that acute stress led to spatial recognition memory impairment in male rats but facilitation in female rats (Conrad et al., 2004). In another study in rats, impairment of recognition memory was found after exposure to stress 30-60 min before the acquisition phase of the object recognition task (Baker and Kim, 2002). Glucocorticoids secreted during acquisition of a stressful task facilitated memory consolidation, whereas acute stress or glucocorticoid treatment, which is not related to the task, impaired performance in mice and rats (e.g. (Klenerova et al., 2002, Dawood et al., 2004)). Chronic stress in rodents has mostly impairing effects on memory and hippocampal function (e.g. (Conrad et al., 2003)).

Animal SD studies have been criticized because SD procedures can be stressful (Siegel, 2001, Vertes, 2004). For example, it has been argued that the “flower pot” method of REM sleep deprivation elicits several secondary

effects which are stressful for the animals. The method is based on muscle atonia typical for REM sleep, which causes animals to fall into the water when they attain REM sleep atonia. They thus, get wet which leads to hypothermia and stress. Moreover, motor activity on the platform is restricted during such SD procedures that may lead to muscle fatigue. The method is not selective for REM sleep, because it is accompanied by a substantial loss of NREM sleep. It has therefore been questioned whether memory impairment observed in sleep-deprived animals can be attributed specifically to sleep loss or rather to the effects of stress. In some studies animals were tested immediately after the termination of SD. Therefore, they might have been sleepy and not motivated. It was unclear whether sleep deprivation-induced impairments were learning and memory deficits or performance deficits. A further important issue is the duration of SD which often exceeds the physiologically reasonable duration.

In the last two papers, we tried to account for these confounding factors and to minimize their influence. Thus, the SD procedure was especially adapted to minimize stress. Animals were sleep deprived by procedures which stimulated their natural behavior (“gentle handling”). The mice and hamsters were continuously observed, and when they assumed a sleep posture, they were disturbed by introducing tissues into the cage or by mild acoustic stimulation (tapping on the cage). Special care was taken not to interfere with feeding and drinking behavior. The duration of SD was limited to 4 h in hamsters and to 6 h in mice. To evaluate the potential contribution of stress induced during SD on recognition memory impairment we measured plasma levels of stress hormones (corticosterone, ACTH and cortisol).

Paper 1

Selective sleep deprivation after daily torpor in the Djungarian hamster

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Published in Journal of Sleep Research, 2002: 11(4), 313-319

SUMMARY
Sleep, daily torpor and hibernation are no longer considered homologous processes. Animals emerging from these states spend most of their time in sleep. After termination of the torpor-associated hypothermia, there is an initial high EEG slow-wave activity (SWA, 0.75-4.0 Hz) and a subsequent monotonic decline. Both of these features are similar to the effects elicited by prolonged waking. It was previously shown that when hamsters are not allowed to sleep immediately after emerging from torpor, an additional SWA increase above the level reached after sleep deprivation (SD) alone occurs during the delayed recovery. A similar manipulation in hibernating ground squirrels abolished the subsequent SWA increase, shedding doubt on the similarity of the regulatory aspects following torpor and hibernation. To further investigate the extent to which SWA is homeostatically regulated after torpor, Djungarian hamsters were subjected to 1.5 h partial nonREM (NREM) sleep deprivation (NSD) that either immediately followed the emergence from torpor (T+NSD) or 4-h SD (SD+NSD). NSD was attained by disturbing the animals when they exhibited NREM sleep with high amplitude slow-waves.

To investigate whether regional aspects of sleep homeostasis are similar after torpor and sleep deprivation, the EEG was recorded from a parietal and frontal derivation after 4-h SD.

An increase in SWA in NREM sleep occurred after all conditions in both EEG derivations. There was no significant difference in SWA during the initial 1.5-h recovery when torpor, T+NSD and SD+NSD were compared. During recovery from torpor and SD, SWA was higher in the frontal than in the parietal derivation.

Our results provide further evidence that torpor and SD have similar effects on sleep. The SWA increase did not disappear after the NSD; therefore, SWA is homeostatically regulated after daily torpor. The frontal predominance of slow waves encountered both after torpor and sleep deprivation indicates that waking and torpor induce similar regional changes in EEG SWA.

KEYWORDS Daily torpor, Djungarian hamster, EEG, Sleep regulation, Sleep deprivation, Topography

INTRODUCTION

Daily torpor and hibernation are behaviours that facilitate survival during winter. Hibernation is a hypometabolic state during which major physiological changes occur in the organism, including a remarkable decrease in body temperature and metabolism (Snapp and Heller 1981). Daily torpor also belongs to the hypothermic states. In contrast to hibernation it lasts only several hours, and the decrease of body temperature to approximately 15-20°C is not as dramatic as the one observed in hibernators (Heldmaier and Ruf 1992).

It is well known that during NREM sleep body temperature is lower compared to quiet waking, and the progressive entrance into a torpor episode is accomplished via NREM sleep (Walker *et al.* 1979). On the basis of these findings, a common energy conserving function was proposed for hibernation, daily torpor and sleep (Horne 1977; Heller *et al*. 1988; Berger 1984).

Animals do not remain continuously torpid throughout the hibernation season, but regularly warm up until they reach the species typical euthermic body and brain temperature, despite the high energetic costs of these arousals. During the initial phase of euthermy high levels of EEG power in NREM sleep in the slow-wave range were found in several species. This slow-wave activity subsequently declined monotonically, with dynamics similar to the well-known changes in SWA after prolonged waking (Daan *et al*. 1991; Trachsel *et al*. 1991). These findings led to the assumption that animals incur a sleep deprivation (SD) during hibernation and daily torpor (Daan *et al*. 1991; Trachsel *et al*. 1991). Moreover, a positive correlation between the slow-wave increase and hypothermic bout duration led to the suggestion that sleep following upon a hibernation bout is homeostatically regulated (Trachsel *et al*. 1991; Strijkstra and Daan 1997b). These results and conclusions were supported by similar experiments in the Djungarian hamster, a species which engages regularly in daily torpor during the winter months (Deboer and Tobler 1996; Deboer and Tobler 2000).

In Djungarian hamsters exposed to 1.5-h SD immediately after they spontaneously aroused from a torpor episode, the initial increase in slow-wave activity (SWA, EEG power density between 0.75-4.0 Hz) in NREM sleep was above the level of SD alone (Deboer and Tobler 2000). This finding supported the notion that sleep occurring after daily torpor episodes is homeostatically regulated and is indistinguishable from sleep following prolonged wakefulness.

In contrast, in ground squirrels SWA (1.0-4.0 Hz or 1.2-4.0 Hz) did not increase when SD was performed during the first 3-4 h immediately following emergence from hibernation. However, a predictable increase of SWA was observed when SD was performed a few hours later (Larkin and Heller 1998; Strijkstra and Daan 1998). This lack of homeostatic regulation of SWA after the combination of hibernation and SD led to the conclusion that the mechanisms underlying the SWA increase in sleep after hibernation are not identical to those leading to its increase after prolonged waking. Moreover, when hibernation occurred at an ambient temperature >15°C, SWA failed to increase compared to the increase observed when hibernation occurred at lower ambient temperatures (Strijkstra and Daan 1997a). It was proposed that the low body and brain temperature during deep hibernation causes loss of neuronal connections (Strijkstra and Daan 1998) and other structures (Larkin

and Heller 1998) and that subsequent return to euthermy allows neuronal recovery and induces slow wave oscillations.

Although it is possible that the different results obtained in ground squirrels and the Djungarian hamster can be attributed to species differences, the effects may have been influenced by the different amount of sleep that occurred during the SD. The ground squirrels slept 13-20% of recording time during the SD (Larkin and Heller 1998; Strijkstra and Daan 1998), whereas the hamsters obtained less than 3 % sleep (Deboer and Tobler 2000). The possibility remains that this "partial" sleep deprivation in the ground squirrels, which allowed considerable sleep to occur, albeit with lower amounts of slow-waves than if sleep had been allowed, could lead to similar results in the hamsters. It has been shown previously that partial sleep deprivation is a powerful tool to investigate mechanisms of sleep homeostasis. In humans and in rats, selective prevention of slow-wave sleep resulted in a marked rebound of slow-waves during recovery (Moses *et al*. 1975; Dijk *et al*. 1987; Endo *et al*. 1997).

We exposed Djungarian hamsters to partial deprivation of NREM sleep (NSD) during the initial phase of arousal from torpor, with the aim of reducing SWA. If sleep homeostasis plays an important role in the regulation of SWA during this phase, we expected a continuing accumulation of sleep pressure, and a postponed manifestation of SWA until sleep is allowed. The effect of torpor followed by NSD was compared with the effect of SD followed by NSD, torpor alone and SD alone in the same animals.

Regional differences occur in EEG power density spectra of mice and rats during recovery from sleep deprivation. A frontal predominance in the slow-wave range was reported (Schwierin *et al.* 1999; Huber *et al*. 2000). The present recordings were based on two cortical EEGs, one overlying the parietal cortex and a second over the frontal cortex. Similar regional differences after SD and daily torpor in the Djungarian hamster would support the hypothesis that sleep pressure accumulates during both of these hypothermic states.

METHODS

Animals

Adult Djungarian hamsters (*Phodopus sungorus*) (n=8; 6 males and 2 females), which had been raised in summer under a natural photoperiod, were kept individually in Macrolon cages (36x20x35 cm) with food and water available ad libitum, and maintained in an 8-h light - 16-h dark cycle (light from 09:00 - 17:00 h; 7 Watt OSRAM DULUX EL energy saving lamp, approximately 30 lux). Mean ambient temperature (T_A) was 14.8±0.2°C and did not differ between 4 of the conditions (baseline, torpor, T+NSD and SD+NSD, ANOVA factor 'day', n.s.), but was significantly higher on the day which included the 4-h SD (16.4±0.3°C, two-tailed paired *t*-test $p<0.01$).

Surgery

When the weight reduction and the fur colour index (index # 1-6; Figala *et al*. 1973) indicated a strong adaptation to the short photoperiod, animals were selected for i.p. implantation of temperature-sensitive transmitters (model X-M, Mini-mitter). At the age of 5.3±0.4 months, when episodes of daily torpor were recognised on the basis of body temperature recordings, the hamsters

(mean weight 26.6±1.3 g) were implanted under deep anaesthesia (Ketalar® 75 mg/kg, Parke-Davis; Rompun® 4 mg/kg, Bayer, i.p.) with gold-plated miniature screws (0.9 mm diameter) that served as EEG electrodes. Screws were placed epidurally over the right parietal cortex (2 mm lateral to midline and 2 mm posterior to bregma), right frontal cortex (2 mm lateral to midline and 2 mm anterior to bregma) and a reference electrode was placed over the cerebellum (2 mm posterior to lambda, on midline). A thermistor (Thermometrics, P20, R (25°C) = 1 kΩ, max. diam. = 0.5mm, accuracy ±0.05°C) was inserted between the skull and dura through a hole over the left frontal cortex (2-3 mm lateral to midline and 2 mm anterior to bregma) to measure cortical temperature (T_{CRT}). Two gold wires inserted into the neck muscles (diameter 0.2 mm) served to record the electromyogram (EMG). The electrodes and thermistor were connected to stainless steel wires that were fixed to the skull with dental cement (Deboer *et al.* 1994). At least one week was allowed for recovery.

Experimental protocol

The EEGs, EMG and T_{CRT} were continuously recorded for several consecutive 24-h days. On a day where torpor occurred that exceeded 4 h, the hamster was either left undisturbed (T), or was subjected to 1.5 h partial deprivation of NREM sleep with high amplitude slow-waves (T+NSD). NSD was achieved based on the EEG amplitude of the polygraph recording. Whenever the hamster was in NREM sleep and high amplitude slow waves were observed, the animal was disturbed by tapping on the cage. The hamsters were never disturbed while they were in torpor or emerging from torpor. Torpor epochs occurred within 7:45 a.m. and 18:40 p.m. In the T+NSD experiment, NSD began when T_{CRT} reached 27°C (Deboer and Tobler 1996). In cases when the manipulations were carried out after lights off, a dim red light (< 1 lux) allowed observation of the animals. A day with no attempt to enter torpor served as baseline (BL). On another non-torpor day the same hamsters were exposed to 4 h SD alone or to 4 h SD followed by 1.5 h NSD (SD+NSD). All treatments were applied in a randomised order, with the exception of SD alone, which was performed when all other conditions had been recorded. All hamsters were subjected to all treatments with the exception of n=2, which were not subjected to the 4-h SD. The day before the 4-h SD alone was used as an additional baseline day. SD began at light onset and consisted of introducing objects into the cage (e.g. nesting material), and later by tapping on the cage, whenever the animal appeared drowsy or attempted to assume a sleeping position. Halfway through the SD the hamsters were provided with a new cage. To minimise stress, some soiled wood chips and nesting material from the old cage was transferred to the new cage. The hamsters were never disturbed during feeding and drinking.

Data acquisition and analysis

The EEG and EMG signals were amplified (amplification factor approx. 2,000), conditioned by analogue filters (high-pass filter: -3 dB at 0.016 Hz; low-pass filter: -3 dB at 40 Hz, less than -35 dB at 128 Hz) sampled with 512 Hz, digitally filtered (EEG: low-pass FIR filter 25 Hz; EMG: band-pass FIR filter 20-50 Hz) and stored with a resolution of 128 Hz. EEG power spectra were computed for 4-s epochs by a Fast Fourier Transform (FFT) routine.

Adjacent 0.25-Hz bins were averaged into 0.5-Hz (0.25 - 5.0 Hz) and 1.0-Hz (5.25-25.0 Hz) bins. The EMG was full-wave rectified and integrated over 4-s epochs, T_{CRT} and T_A inside the cage were sampled at 4-s intervals. Before each recording the EEG and EMG channels were calibrated with a 10 Hz sine wave, 300 μV peak to peak signal.

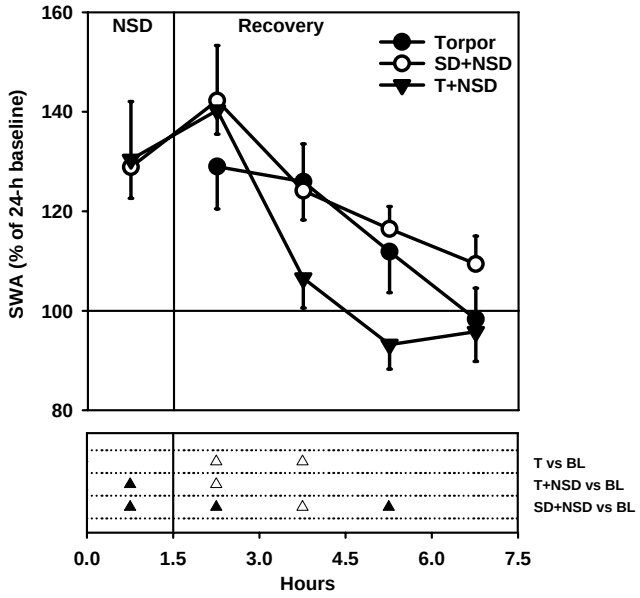

Figure 1
Time course of slow-wave activity (SWA, mean EEG power density 0.75-4.0 Hz) in non-rapid eye movement (NREM) sleep during recovery after torpor alone (Torpor, T), sleep deprivation followed by 1.5-h NREM sleep deprivation (SD+NSD) and torpor followed by 1.5-h NSD (T+NSD)**.** Mean SWA for 1.5-h intervals ±SEM (n=8) are expressed relative to the mean 24-h baseline (BL=100%). Two-way rANOVA factor 'time: 1.5-h interval of recovery', $p<0.0001$; 'condition: torpor, T+NSD, SD+NSD', n.s., interaction: 'condition' x 'time', n.s.). Significant differences between baseline and conditions are indicated by triangles ($p<0.05$ white, $p<0.01$ black, two-tailed paired t-test). The vertical line delimits the 1.5-h NSD.

The three vigilance states NREM sleep, REM sleep and waking were scored for 4-s epochs as in previous studies (Deboer *et al*. 1994; Deboer and Tobler 1996). Vigilance states were determined off-line by visual inspection of the parietal and frontal EEG and EMG records and EEG power in the slow-wave range (0.75-4.0 Hz). Epochs containing EEG artifacts in one derivation were excluded from spectral analyses of both EEG derivations (12.0±0.8 SEM percentage of recording time, which occurred mainly during active waking). Vigilance states could always be determined.

For analysis, recovery was subdivided into 1.5-h intervals to match the duration of the additional NSD intervention. Time intervals of each condition were matched with corresponding baseline intervals within individuals. The comparison between the conditions was based on a maximum of 7.5 h after the end of torpor or SD.

Differences in EEG spectra between treatment days were tested with two-way ANOVA for repeated measures (rANOVA), factors 'condition: baseline, torpor, T+NSD, SD+NSD or SD' and 'time: interval'. Whenever significant effects were present ($p<0.05$) further differences were evaluated by post hoc two-tailed paired *t*-tests.

RESULTS

During baseline, the amount of NREM sleep and REM sleep did not vary over 24 h ($p>0.11$, one-way ANOVA on 1.5 h intervals, not shown). The duration of torpor did not differ significantly between the torpor alone and T+NSD condition (6.50±0.52 h ± SEM and 6.56±0.54 h, respectively; two-tailed paired *t*-test).

Table 1

	Corresponding baseline interval				Recovery after conditions			
	NREMS (%)	REMS (%)	Waking (%)	NREM T_{CRT} (°C)	NREMS (%)	REMS (%)	Waking (%)	NREM T_{CRT} (°C)
Torpor :	37.1	7.4	55.5	34.7	69.3	8.1	22.6	33.8
0-1.5 h	(6.3)	(1.8)	(7.8)	(0.2)	(3.7)*	(1.3)	(4.4)	(0.3)***
1.5-3.0 h	44.3	7.7	48.0	35.0	51.7	8.1	40.2	35.3
	(4.8)	(1.4)	(5.8)	(0.2)°	(3.4)	(1.0)	(3.9)	(0.3)
3.0-4.5 h	49.0	8.6	42.4	35.1	48.4	10.3	41.3	34.9
	(6.5)	(1.9)	(8.3)	(0.2)	(4.4)	(1.3)	(4.5)	(0.3)
4.5-6.0 h	46.8	8.6	44.6	35.0	39.7	6.7	53.6	35.2
	(8.2)	(2.2)	(10.2)	(0.2)	(5.0)*	(1.6)	(6.5)	(0.2)
Total 6 h	44.3	8.1	47.6	34.9	52.3	8.3	39.4	34.7
	(2.3)	(0.7)	(2.6)	(0.2)	(1.6)*	(0.9)	(1.9)	(0.3)
T+NSD:	44.2	7.8	48.0	35.0	31.4	1.0	67.6	34.9
NSD	(5.3)	(1.1)	(6.2)	(0.2)°	(5.5)	(0.6)	(6.0)*	(0.5)§
0-1.5 h	49.6	8.7	41.7	34.9	56.5	10.2	33.3	34.9
	(7.1)	(1.9)	(8.8)	(0.2)	(5.6)	(1.9)	(6.6)	(0.3) †
1.5-3.0 h	37.9	6.6	55.5	35.2	51.3	11.1	37.6	35.1
	(7.0)	(1.9)	(8.5)	(0.2)°	(2.9)	(1.4)	(4.0)	(0.2)
3.0-4.5 h	51.1	8.8	40.1	35.0	42.2	8.7	49.1	34.5
	(5.1)	(1.4)	(6.2)	(0.2)	(3.3)	(0.8)	(3.6)	(0.6)
4.5-6.0 h	47.7	7.7	44.6	34.8	47.9	9.0	43.1	35.2
	(7.8)	(1.8)	(9.5)	(0.2)	(7.0)	(2.1)	(8.6)	(0.2)
Total 6 h	46.6	7.9	45.5	34.9	49.5	9.7	40.8	35.0
	(3.2)	(0.7)	(3.7)	(0.2)	(2.3)	(0.8)	(2.7)	(0.5)
SD+NSD:	50.4	9.7	40.0	34.5	37.5	0.4	62.1	36.0
NSD	(4.7)	(0.9)	(5.2)	(0.2)	(4.2)**	(0.2)***	(4.3)***	(0.3)***
0-1.5 h	56.0	11.4	32.6	34.6	58.9	11.2	29.9	35.2
	(4.7)	(1.3)	(4.7)	(0.2)	(2.8)	(0.5)	(2.9)	(0.3) †
1.5-3.0 h	48.0	7.8	44.2	34.8	48.3	11.4	40.3	35.0
	(4.6)	(1.1)	(5.7)	(0.2)	(4.5)	(1.2)	(4.9)	(0.3)
3.0-4.5 h	41.9	6.9	51.2	34.9	51.7	9.2	39.1	35.0
	(6.6)	(1.7)	(8.1)	(0.2)	(6.0)	(1.9)	(7.4)	(0.2)
4.5-6.0 h	42.7	8.0	49.3	35.1	47.3	10.8	41.9	35.0
	(8.2)	(2.0)	(10.0)	(0.3)	(4.8)	(1.5)	(6.1)	(0.3)
Total 6 h	47.2	8.5	44.3	34.8	51.6	10.6	37.8	35.0
	(1.6)	(0.6)	(1.4)	(0.2)	(1.4)	(0.6)*	(1.8)	(0.3)

Mean values (SEM in parenthesis, n=8) of waking, non-rapid eye movement (NREM) sleep, REM sleep and cortical temperature (T_{CRT}) in NREM sleep for 1.5-h and 6-h intervals (Total 6 h) during recovery after torpor, and during 1.5-h NREM sleep deprivation (NSD) and subsequent recovery (last 4 data points) after sleep deprivation (SD+NSD) and torpor (T+NSD) and corresponding baseline intervals. Time intervals of each condition were matched with corresponding baseline intervals within individuals. The comparison between the conditions was based on a maximum of 7.5 h after the end of torpor or SD. Vigilance states are expressed as percentage of recording time. Significant differences between corresponding baseline intervals of Torpor or T+NSD and SD+NSD (°$p<0.05$, two-tailed paired t-test), effect of manipulations (*$p<0.05$, **$p<0.01$, ***$p<0.005$), difference between either T+NSD or SD+NSD and Torpor ($^{\dagger}p<0.01$) and difference between T+NSD and SD+NSD ($^{\S}p<0.05$) are indicated.

All conditions induced a significant initial increase of SWA in NREM sleep with the exception of the first 30 min after torpor alone (the values for the first 1.5-h interval after torpor was 129.0±8.5% of the 24-h baseline and after SD alone 156.1±5.3%; Figs. 1 and 3). The SWA increase did not differ significantly between the three conditions illustrated in Figure 1 (rANOVA factor: 'interval' $p<0.0001$, 'condition' n.s.). SWA was significantly above the

corresponding baseline level after all conditions. Significance was reached in the first 1.5 h after T+NSD, while after torpor alone, SD+NSD and 4-h SD the SWA increase lasted for 3 h, 4.5 h and 4.5 h, respectively (two-way rANOVA resulted in no significant differences in time course: interaction factors 'condition' torpor, T+NSD and SD+NSD x 'time' 1.5-h interval).

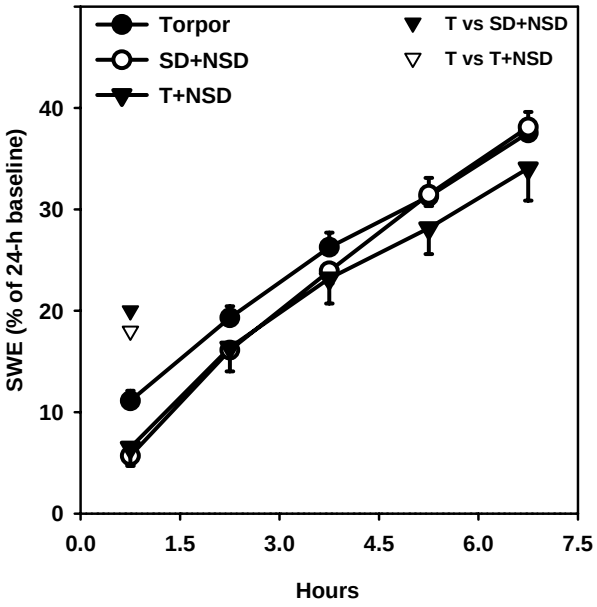

Figure 2
Time course of slow-wave energy (SWE; mean EEG power density between 0.75-4.0 Hz) in non-rapid eye movement (NREM) sleep during recovery after torpor alone (Torpor), during 1.5-h NREM sleep deprivation (first value) and subsequent recovery (remaining 4 values) after sleep deprivation (SD+NSD) and torpor (T+NSD). Curves connect 1.5-h cumulative values of mean SWE (±SEM, n=8) which are expressed relative to the mean 24-h baseline value. Triangles: significant differences between conditions ($p<0.05$ white, $p<0.001$ black, two-tailed paired t-test).

The vigilance states during recovery did not differ between the conditions torpor alone, T+NSD and SD+NSD (Table 1). NREM sleep was higher after torpor during several 1.5-h intervals and over the entire initial 6-h recovery interval, compared to the corresponding time intervals of baseline.
During the additional intervention (NSD) waking was increased above baseline during both NSDs (Table 1). The amounts of NREM sleep, REM sleep and waking did not differ significantly between the two NSD's. In addition, SWA in NREM sleep during the NSD was reduced to a similar extent in T+NSD and SD+NSD (Fig. 1).

To investigate the relationship between sleep duration (TST) and sleep intensity (SWA) during recovery, slow-wave energy (SWE, i.e. cumulative values of EEG power density between 0.75-4.0 Hz in NREM sleep) was computed (Fig. 2). SWE immediately after torpor was significantly higher than during either of the NSD's (when values of the 1.5 h after the interventions were computed as % of the first 1.5 h after torpor, SD+NSD was 51.4±6.4 % and T+NSD 61.0±19.7 %), indicating that the "partial" SD was successful in reducing SWE by approximately 40-50%. Thereafter, no differences in SWE were observed between the three conditions (Fig. 2).

Cortical temperature during the NSD intervention was significantly higher in the SD+NSD condition compared to T+NSD (Table 1). During recovery, T_{CRT} in NREM sleep was significantly lower in the first 1.5 h after torpor compared to the corresponding interval after T+NSD or SD+NSD and compared to T_{CRT} during both NSDs. During the remaining hours no further differences occurred between the three conditions.

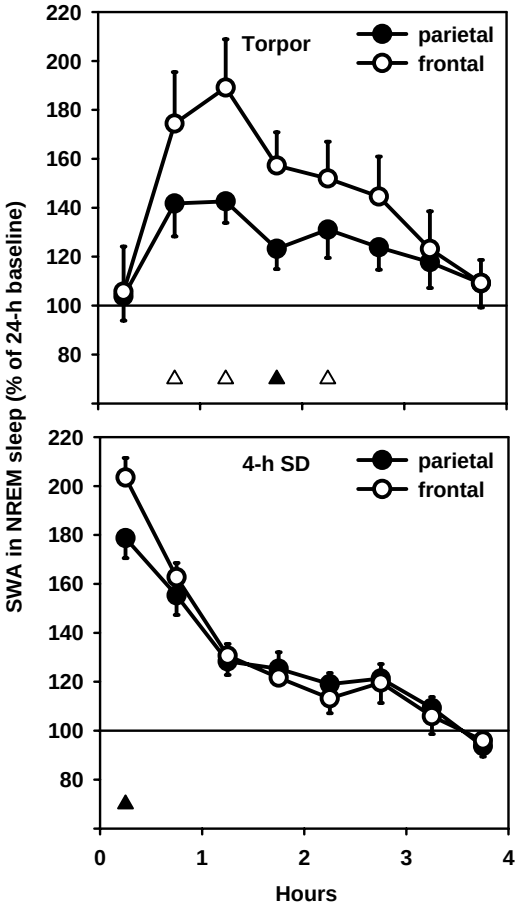

Figure 3
Time course of slow-wave activity (SWA, mean EEG power density 0.75-4.0 Hz) in non-rapid eye movement (NREM) sleep of the parietal and frontal derivation during recovery after torpor alone (n=8; top panel) and after 4-h sleep deprivation (SD, n=6; bottom panel). Mean SWA values ±SEM are expressed relative to the mean 24-h baseline value (baseline 1 for torpor and baseline 2 for SD; baseline 1 vs baseline 2 were n.s.) of the corresponding derivation (=100%). Significant differences between derivations are indicated by triangles (rANOVA factor '30-min interval' x 'derivation': $p<0.01$; $p<0.05$ white, $p<0.01$ black, two-tailed paired t-test).

In several species topographic differences in SWA were observed when the animals slept under higher sleep pressure after SD (Schwierin *et al.* 1999; Huber *et al*. 2000). We therefore compared the effect of SD and of torpor on the frontal and parietal EEG in the hamsters. A significant frontal predominance in the mean 24-h EEG power spectrum in NREM sleep was present in baseline in the range between 1.25-3.0 Hz compared to the parietal derivation (data not shown). After both SD and torpor the increase of SWA in NREM sleep was significantly larger in the frontal derivation, than in the parietal derivation (Fig. 3). The fronto-parietal difference was restricted to the first 30 min of recovery after SD, whereas after torpor it lasted 2 h.

DISCUSSION

The main finding was the persistence of an initial SWA increase after the additional NSD performed immediately after daily torpor. Moreover, the SWA increase was similar when the NSD was performed after 4 h SD. This result is in accordance with the hypothesis that sleep pressure is increased after daily torpor and needs to be dissipated during subsequent sleep (Deboer and Tobler 1994; Deboer and Tobler 1996; Deboer and Tobler 2000).

SWA in NREM sleep is thought to reflect sleep intensity (Borbély 1982; Daan *et al*. 1984). SWA increases as a function of the duration of prior spontaneous or induced waking and decreases progressively when sleep is allowed (Tobler and Borbély 1990; Huber *et al*. 1999). A positive correlation had been obtained previously between duration of the torpor episodes and subsequent SWA during sleep. Moreover, the correlation was characterised by

a saturating exponential curve (Deboer and Tobler 1996), implying that there is a similar kinetics of build-up of sleep pressure during daily torpor and waking.

In the rat, selective NREM sleep deprivation, tested in euthermic conditions, proved to be an effective tool to maintain sleep pressure until recovery was allowed (Endo *et al.* 1997). Similarly, in the Djungarian hamster, the NSD maintained the level of SWA in NREM sleep, which was manifested subsequently at a similar SWA level after T+NSD and SD+NSD. The selective intervention diminished SWE by 40-50% compared to torpor alone, and the magnitude of the reduction did not significantly differ between the two interventions. After both manipulations, the initial SWA was slightly but non-significantly above the NSD values, an effect that is probably a consequence of the waking induced by the manipulation. These results further support the notion that the mechanisms by which sleep debt accumulates after daily torpor are similar to the mechanisms leading to the build-up of SWA during prolonged waking, despite large physiological differences between these states.

After an initial peak, SWA decreased more rapidly after T+NSD than after torpor alone or SD+NSD, although the rANOVA did not result in significant differences. Moreover, the amount of NREM sleep did not differ between the conditions. It is important that a similar amount of slow wave energy was recovered in the course of the first 6 hours.

To investigate further potential similarities between torpor and SD the topographic effects on the sleep EEG were compared after both interventions. A frontal predominance of the initial SWA increase occurred after arousal from torpor as well as after SD (Fig. 3). A frontal predominance after SD was previously observed in rats (Schwierin *et al*. 1999), two mouse strains (Huber *et al*. 2000), and in humans (Cajochen *et al.* 1999), and has now been confirmed in hamsters. It has been suggested that regional EEG differences during sleep may reflect differences in neuronal activity occurring during preceding waking (Kattler *et al.* 1994; Vyazovskiy *et al.* 2000), supporting the hypothesis that sleep has local, use-dependent features (Krueger and Obál 1995).

On the other hand, it has been proposed that the function of sleep is to stimulate synapses that were insufficiently used during waking and to maintain neuronal connections (Krueger and Obál 1993; Kavanau 1999). It was suggested that the need to restore synaptic efficacy is the basis for periodic returns to euthermic sleep (Kavanau 1997). Several morphological changes in synapses observed in the middle of a hibernation bout, as well as their reversal within the first 2 h in euthermia support this view (Popov *et al*. 1992; Popov and Bocharova 1992). After torpor the frontal SWA predominance, a feature seen previously in humans, rats and mice after SD, lasts longer than after the 4-h SD. This difference may indicate that during the inactive state of torpor, changes occur which have a relatively large influence on this parameter, supporting the hypothesis that a function of sleep could be the stimulation of synapses insufficiently used.

In summary, the results show that SWA after daily torpor is homeostatically regulated. Sleep debt accumulates during torpor as it does during prolonged waking. A partial sleep deprivation aimed at reducing SWA after termination of torpor led to an increase in SWA, as predicted by the two-process model of sleep regulation. A predominance of SWA in the frontal EEG

was observed after both SD and torpor, adding a further similarity between these two conditions.

ACKNOWLEDGEMENTS

We thank Dr. G. Heldmayer for providing us with the hamsters, Dr. Caroline Kopp, Vlad Vyazovskiy and Stephanie Fauser for technical assistance during the manipulations, and Dr. J. Gottselig for critical reading of the manuscript. The study was supported by the Swiss National Science Foundation grant nr. 3100-053005.97/2.

REFERENCES

Borbély A.A. A two process model of sleep regulation. *Hum. Neurobiol.*, 1982, 1: 195-204.

Berger, R. J. Slow wave sleep, shallow torpor and hibernation: homologous states of diminished metabolism and body temperature. *Biol. Psychol.*, 1984, 19: 305-326.

Cajochen, C., Foy, R. and Dijk, D.J. Frontal predominance of a relative increase in sleep delta and theta EEG activity after sleep loss in humans. *Seep Res. Online*, 1999, 2: 65-69.

Daan, S., Beersma, D.G.M. and Borbély, A.A. Timing of human sleep: recovery process gated by a circadian pacemaker. *Am. J. Physiol*., 1984, 246: 161-183.

Daan, S., Barnes, B.M. and Strijkstra, A.M. Warming up for sleep? - ground squirrels sleep during arousals from hibernation. *Neurosci Lett.*, 1991, 128: 265-268.

Deboer, T., Franken, P. and Tobler, I. Sleep and cortical temperature in the Djungarian hamster under baseline conditions and after sleep deprivation. *J. Comp. Physiol. A*, 1994, 174: 145-155.

Deboer, T. and Tobler, I. Sleep EEG after daily torpor in the Djungarian hamster: similarity to the effects of sleep deprivation. *Neurosci. Lett*., 1994, 166: 35-38.

Deboer, T. and Tobler, I. Natural hypothermia and sleep deprivation: common effects on recovery sleep in the Djungarian hamster. *Am. J. Physiol*., 1996, 271: R1364-R1371.

Deboer, T. and Tobler, I. Slow waves in the sleep electroencephalogram after daily torpor are homeostatically regulated. *NeuroReport*, 2000, 2: 881-885.

Dijk, D.J., Beersma, D.G.M. and Daan, S. EEG power density during nap sleep: reflection of an hourglass measuring the duration of prior wakefulness. *J. Biol. Rhythms*, 1987, 2: 207-219.

Endo, T., Schwierin, B., Borbély, A.A. and Tobler, I. Selective and total sleep deprivation: effect on the sleep EEG in the rat. *Psych. Res*., 1997, 66: 97-110.

Figala, J., Hoffmann, K. and Goldau, G. Zur Jahresperiodik beim Dsungarischen Zwerghamster *Phodopus sungorus Pallas*. *Oecologia,* Berlin, 1973, 12: 89-118.

Heldmaier, G. and Ruf, T. Body temperature and metabolic rate during natural hypothermia in endotherms. *J. Comp. Physiol. B*, 1992, 162: 696-706.

Heller, H.C. Sleep and hypometabolism. *Can. J. Zool*., 1988, 66: 61-69.

Horne, J. A. Factors relating to energy conservation during sleep in mammals. *Physiol. Psychol*., 1977, 5: 403-408.

Huber, R., Deboer, T. and Tobler, I. Prion protein: a role in sleep regulation? *J. Sleep Res.,* 1999, 8: 30-36.

Huber, R., Deboer, T. and Tobler, I. Topography of EEG dynamics after sleep deprivation in mice. *J. Neurophysiol*., 2000, 84: 1888-1893.

Kattler, H., Dijk, D.J. and Borbély, A.A. Effect of unilateral somatosensory stimulation prior to sleep on the sleep EEG in humans. *J. Sleep Res*., 1994, 3: 159-164.

Kavanau, J.L. Memory, sleep and the evolution of mechanisms of synaptic efficacy maintenance. *Neurosci*., 1997, 79: 7-44.

Kavanau, J.L. Adaptations and pathologies linked to dynamic stabilization of neural circuitry. *Neurosci. Biobehav. Rev*., 1999, 23: 635-648.

Krueger, J.M. and Obál, F. A neuronal group theory of sleep function. *J. Sleep Res.*, 1993, 2: 63-69.

Krueger, J.M., Obál, F. J., Kapàs L. and Fang J. Brain organization and sleep function. *Behav. Brain Res*., 1995, 69: 177-185.

Larkin, J.E. and Heller, H.C. The disappearing slow wave activity of hibernators. *Sleep Research Online*, 1998, 1: 96-101.

Moses, J.M., Johnson, L.C., Naitoh, P. and Lubin, A. Sleep stage deprivation and total sleep loss: effects on sleep behaviour. *Psychophys*., 1975, 12: 141-146.

Popov, V.I. and Bocharova, L.S. Hibernation-induced structural changes in synaptic contacts between mossy fibres and hippocampal pyramidal neurones. *Neurosci*., 1992, 48: 53-62.

Popov, V.I., Bocharova, L.S. and Bragin, A.G. Repeated changes of dendritic morphology in the hippocampus of ground squirrels in the course of hibernation. *Neurosci*., 1992, 48: 45-51.

Schwierin, B., Achermann, P., Deboer, T., Oleksenko, A., Borbély, A.A. and Tobler, I. Regional differences in the dynamics of the cortical EEG in the rat after sleep deprivation. *Clin. Neurophysiol*., 1999, 110: 869-875.

Snapp, B. D. and Heller, H. C. Suppression of metabolism during hibernation in ground squirrels (*Citellus lateralis*). *Physiol. Zool*., 1981, 54: 297-307.

Strijkstra, A.M. and Daan, S. Ambient temperature during torpor affects NREM sleep EEG during arousal episodes in hibernating European ground squirrels. *Neurosci. Lett*., 1997a, 221: 177-180.

Strijkstra, A.M. and Daan, S. Sleep during arousal episodes as a function of prior torpor duration in hibernating European ground squirrels. *J. Sleep Res*., 1997b, 6: 36-43.

Strijkstra, A.M. and Daan, S. Dissimilarity of slow-wave activity enhancement by torpor and sleep deprivation in a hibernator. *Reg. Integr. Comp. Physiol*., 1998, 44: R1110-R1117.

Tobler, I. and Borbély, A.A. The effect of 3-h and 6-h sleep deprivation on sleep and EEG spectra of the rat. *Behav. Brain Res*., 1990, 36: 73-78.

Trachsel, L., Edgar, D.M. and Heller, H.C. Are ground squirrels sleep deprived during hibernation? *Am. J. Physiol*., 1991, 260: R1123-R1129.

Vyazovskiy, V., Borbély, A.A. and Tobler, I. Unilateral vibrissae stimulation during waking induces interhemispheric EEG asymmetry during subsequent sleep in the rat. *J. Sleep Res.*, 2000, 9: 367-371.

Walker, J.M., Garber, A. Berger, R.J. and Heller, H.C. Sleep and estivation (shallow torpor): continuous processes of energy conservation. *Science,* 1979, 204: 1098-1100.

Paper 2

Seasonal aspects of sleep in the Djungarian hamster

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Published in BMC Neuroscience 2003: 4, 9

Abstract
Background: Changes in photoperiod and ambient temperature trigger seasonal adaptations in the physiology and behaviour of many species, including the Djungarian hamster. Exposure of the hamsters to a short photoperiod and low ambient temperature leads to a reduction of the polyphasic distribution of sleep and waking over the light and dark period. In contrast a long photoperiod enhances the daily sleep-wake amplitude leading to a decline of slow-wave activity in NREM sleep within the light period. It is unknown whether these changes can be attributed specifically to photoperiod and/or ambient temperature, or whether endogenous components are contributing factors. The influence of endogenous factors was investigated by recording sleep in Djungarian hamsters invariably maintained at a low ambient temperature and fully adapted to a short photoperiod. The second recording was performed when they had returned to summer physiology, despite the maintenance of the ‘winter’ conditions. Results: Clear winter-summer differences were seen in sleep distribution, while total sleep time was unchanged. A significantly higher light-dark cycle modulation in NREM sleep, REM sleep and waking was observed in hamsters in the 'summer physiological state compared to those in the winter state. Moreover, only in summer, REM sleep episodes were longer and waking bouts were shorter during the light period compared to the dark period. EEG power in the slow-wave range (0.75-4.0 Hz) in both NREM sleep and REM sleep was higher in animals in the summer physiological state than in those in the winter state. In winter SWA in NREM sleep was evenly distributed over the 24 h, while in summer it decreased during the light period and increased during the dark period. Conclusion: Endogenous changes in the organism underlie the differences in sleep-wake redistribution we have observed previously in hamsters recorded in a short and long photoperiod.

Keywords: EEG, Djungarian hamster, seasonality, sleep, spectral analysis, photoperiod

Background

Changes in photoperiod trigger seasonal adaptations in physiology and behaviour of many species [1]. The adaptations are manifold and include changes in body weight, pelage colour and density, altered social and sexual behaviors, in many rodents gonadal regression and suppression of breeding, and in some species hibernation or episodes of daily torpor. In mammals, the main trigger for these changes is the shortening of the photoperiod that determines the duration of pineal melatonin secretion [1-11]. Seasonal changes in physiology can be facilitated by lowering of ambient temperature (T_A) [12-15]. The encoding of seasonal time may involve clock genes, as suggested by the elevated expression of Per1 and Per2 genes in the SCN and pars tuberalis under a long photoperiod [16-19].

Few studies have addressed seasonal changes in sleep. Walker et al [20] reported annual changes in sleep in 4 golden mantled ground squirrels, with the largest amount of sleep in winter. Changes in sleep duration were found in 4 captive prosimians, *Microcebus murinus*, with major reductions of sleep in summer [21]. In humans, under natural conditions in winter and under controlled short photoperiod conditions in the laboratory, sleep duration is increased when nights are long [22-24], but also changes restricted to timing and not duration of sleep have been reported [25]. A behavioural study in elephants in captivity found an increase in sleep duration during the winter months [26]. In contrast, in small rodents, including the rat, Siberian chipmunk, and Djungarian hamster, sleep duration was not affected by a change in photoperiod [27-31]. However, a marked redistribution of sleep occurred across 24 h when the animals were recorded in a long and a short photoperiod.

Figure 1: Djungarian hamsters displaying the typical winter and summer pelage.

The Djungarian hamster is a rodent that typically displays a large spectrum of behavioural and physiological adaptations to changes in photoperiod [2] (for references see [30]). The most prominent change in sleep was the enhancement of the light-dark amplitude in the amount of sleep when the hamsters were adapted to a 'summer' conditions by a long photoperiod, after living in the 'winter' short photoperiod. Moreover, EEG power density in the slow-wave range was lower in the short photoperiod [32]. It is unknown whether the differences in sleep between the two photoperiods are a direct consequence of the change in the environment or if an endogenous component is involved. Syrian hamsters maintained in short days or in constant darkness do not sustain gonadal regression indefinitely, indicating that an endogenous component contributes to these changes [33, 34]. In Djungarian hamsters the amplitude and duration of melatonin secretion returned to summer values despite the maintenance of the short photoperiod [8], (A. Stieglitz, PhD thesis, 1995), and we have frequently observed that the hamsters maintained in a short photoperiod and low T_A nevertheless gain weight and show the typical pelage change from white to dark brown (unpublished; Figure 1). It thus seems that the hamsters undergo a refractory period, despite the maintenance of 'winter' conditions in the environment. We investigated the endogenous nature of changes in sleep by comparing hamsters recorded in winter with animals recorded several months later, when they showed adaptations to summer, while they remained in a short photoperiod and low T_A throughout the experiment.

Table 1: Seasonal differences in the vigilance states.

	Hamsters in winter physiological state			Hamsters in summer physiological state		
	8-h L	16-h D	24 h	8-h L	16-h D	24 h
Waking	23.15 ± 0.94*°	27.43 ± 0.59°	624.03 ± 8.03	19.45 ± 0.90**	30.29 ± 0.81	640.16 ± 15.01
NREMS	31.30 ± 0.90	29.17 ± 0.57°	702.13 ± 9.99	33.69 ± 0.93**	27.21 ± 0.48	685.50 ± 14.80
REMS	5.70 ± 0.33*°	5.00 ± 0.18°°	113.35 ± 4.58	6.93 ± 0.31**	4.35 ± 0.11	114.34 ± 2.89

Mean values ± SEM in min/h in the light (L) and dark (D) period (LD 8:16 h) and 24-h values (minutes) for hamsters in winter physiological state (n=10) and summer physiological state (n=11). Differences between the corresponding periods in winter vs. summer: °p<0.05, °°p<0.005, two-tailed unpaired *t*-test. For n=6, p<0.05 for REM sleep in the light and dark period and waking in the light period; Differences L vs. D: *p<0.05, **p<0.0001, two-tailed paired *t*-test. For n=6, L vs. D in summer, p<0.001 for all vigilance states, two-tailed paired *t*-test).

Results

In summer, when the hamster's pelage was dark brown (Figure 1), they showed a significant increase in the light-dark amplitude of total sleep time, NREM sleep and REM sleep compared to winter, when the fur was white with the typical dark band on the back (Figure 2). The total amount of sleep (TST) and the amounts of the single vigilance states remained at a similar level in winter and summer (Figure 2, Table 1). This result applied also to those hamsters which were recorded in both conditions (n=6; TST: 58.4 ± 2.1% in

winter physiology and 57.9 ± 1.9% in summer physiology; NREM sleep: 50.6 ± 1.8% and 49.6 ± 1.6%; REM sleep: 8.5 ± 0.4% and 8.9 ± 0.4%, paired *t*-test, n.s.). The increase in amplitude in summer was due to a significant increase of TST and a concomitant increase in REM sleep in the light period, while an opposite change occurred in the dark period (Table 1).

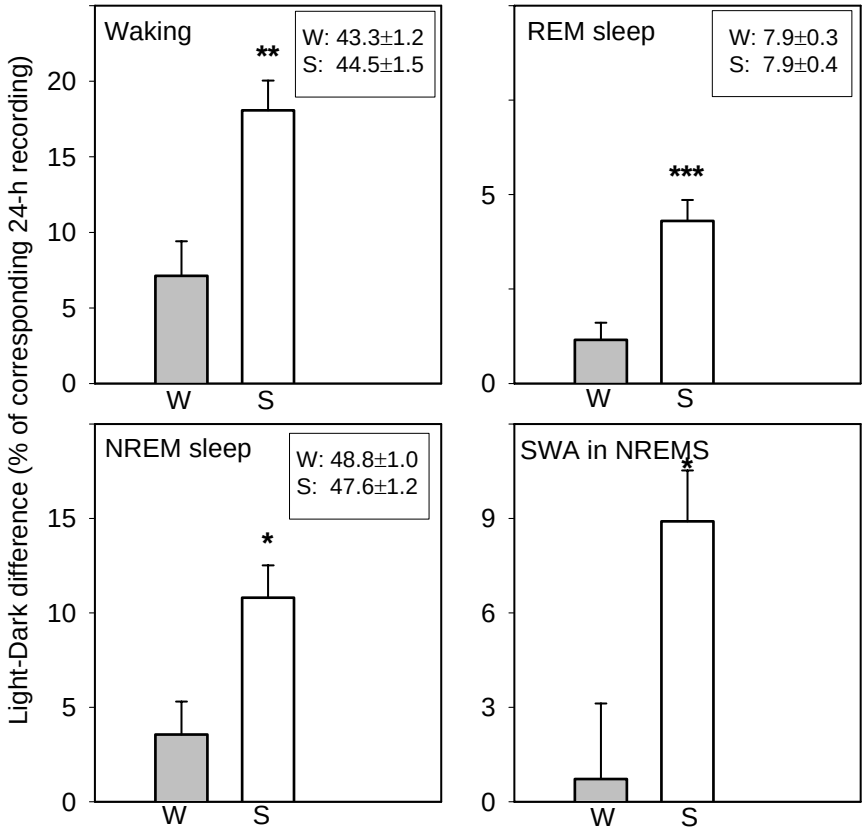

Figure 2: Winter-Summer difference in vigilance-state distribution.
Light-dark differences in the vigilance states and slow-wave activity (SWA, mean EEG power density 0.75-4.0 Hz) in hamsters in physiological state belonging to winter and to summer. Mean 8-h and 16-h values (± SEM) expressed as percentage of the corresponding 24-h for waking, non-rapid eye movement (NREM) sleep and REM sleep. SWA in NREM sleep is expressed relative to the corresponding 24 h mean (=100%). Numbers within panels represent total amount in % of 24 h (mean ±SEM). Winter (W, n=10) and summer (S, n=11). Note different scales of panels. Winter vs. summer: *p<0.01, **p<0.005, ***p<0.0005; unpaired t-test (for n=6: waking p<0.05, NREM sleep p=0.065, REM sleep p<0.01 and SWA p=0.063, paired *t*-test).

The changes in the vigilance states between the two conditions were reflected in the duration and frequency of episodes (Table 2). While in hamsters manifesting winter physiology, episode duration and episode frequency in NREM sleep and waking did not differ between the light and the dark period, and REM sleep episode frequency was larger in the light period compared to the dark period, when recorded in summer physiology, REM sleep episodes were longer, waking episodes shorter, and NREM and REM sleep episodes more frequent in the light period compared to the dark period. None of the 24-h values differed significantly between the hamsters in the winter or in the summer physiological state.

SWA in NREM sleep reflected the redistribution of TST in the light and dark period between the conditions. Thus, the LD amplitude increased significantly from winter to summer (Figure 2). No LD-change in SWA in NREM sleep was observed in winter, while an increase of SWA was seen from the light to the dark period in summer. Within the SWA band, frequencies between 0.75-3.0 Hz increased significantly from winter to summer physiology (all hamsters: 378.07 ± 34.89 μV^2 in winter and 537.67 ± 38.81 μV^2 in summer, unpaired t-test, $p<0.01$; n=6: 386.05 ± 26.93 μV^2 in winter and 493.96 ± 34.95 μV^2 in summer, paired t-test, $p=0.068$). An increase in EEG power was present in frequencies between 1.25 - 2.5 Hz also in REM sleep (not shown). Total EEG power density (0.75-20.0 Hz) over all vigilance states did not differ between the hamsters in winter physiology and summer physiology (1368.34 ± 130.98 μV^2 and 1650.48 ± 119.51 μV^2, n=10 and 11, respectively, unpaired t-test, $p=0.13$; 1368.03 ± 128.19 μV^2 and 1513.63 ± 90.26 μV^2, n=6, paired t-test, $p=0.35$).

Table 2: Seasonal differences in duration and frequency of vigilance state episodes.

	Hamsters in winter physiological state			Hamsters in summer physiological state		
	8-h L	16-h D	24 h	8-h L	16-h D	24 h
Episode duration (min)						
Waking	6.35 ± 0.72	6.27 ± 0.47	6.21 ± 0.42	5.26 ± 0.47**	7.57 ± 0.81	6.75 ± 0.62
NREM sleep	7.54 ± 0.31	7.39 ± 0.38	7.43 ± 0.35	7.94 ± 0.36	7.15 ± 0.31	7.40 ± 0.26
REM sleep	1.73 ± 0.07	1.67 ± 0.07	1.69 ± 0.06	2.08 ± 0.17***	1.64 ± 0.09	1.82 ± 0.12
Episode frequency (# per h)						
Waking	4.15 ± 0.40	4.29 ± 0.29	4.25 ± 0.30	3.98 ± 0.25	4.20 ± 0.41	4.13 ± 0.34
NREM sleep	4.48 ± 0.19	4.15 ± 0.23	4.26 ± 0.19	4.56 ± 0.19*	3.90 ± 0.23	4.12 ± 0.19
REM sleep	3.29 ± 0.20***	2.65 ± 0.15	2.86 ± 0.15	3.52 ± 0.27****	2.36 ± 0.14	2.75 ± 0.16

Mean values (± SEM) for duration (minutes) and frequency (number of episodes per hour) of vigilance state episodes for the light (L) and dark (D) period (LD 8:16 h), and for the entire 24 h recording in the winter physiological state (n=10) and summer physiological state (n=11). L vs. D: *p<0.05, **p<0.01, ***p<0.005, ****p<0.001, two-tailed paired *t*-test. For n=6: 24-h REM sleep episode duration: winter vs. summer, p<0.05, L vs. D in summer, p<0.01; REM sleep episode frequency: L vs D p<0.05 both in winter and summer.

The comparison of the 24-h EEG power spectrum between the two cortical derivations showed a frontal predominance in NREM sleep in the low frequency range both in the hamsters in winter physiology (1.25-2.5 Hz) and summer physiology (1.25-4.0). The difference between the derivations remained the same in the winter and summer physiology hamsters.

To establish whether small differences in T_A or in brain temperature could have contributed to the differences in sleep, several correlations (Pearson product-moment correlation) were computed. No significant correlation was found between brain temperature and EEG parameters (total EEG power: r^2=0.005, p=0.77, n=20, brain temperature data of one hamster were lacking; r^2=0.25, p=0.10, n=12; LD SWA difference, r^2=0.05, p=0.34, n=20 and r^2=0.28, p=0.08 n=12; LD difference in the amount of NREM sleep, REM sleep and waking (n.s., not shown)). Also the correlations with T_A were not significant (total EEG power, r^2=0.03, p=0.50, n=20 and r^2=0.02, p=0.64 n=12; LD SWA difference, r^2=0.12, p=0.132, n=20 and r^2=0.14, p=0.24, n=12; LD difference in the amount of NREM sleep, REM sleep and waking (n.s., not shown)).

Discussion

The main change in sleep observed in hamsters recorded in a physiological state typical for winter and for summer was an enhancement of the sleep-wake amplitude. The total amount of vigilance states remained unchanged, while the polyphasic sleep-wake pattern was redistributed. These summer-winter differences are similar to those observed in hamsters recorded after a prolonged adaptation to a short photoperiod at 16°C or after adaptation to a long photoperiod at 22°C [31] or in hamsters that remained always at 14.5°C but were adapted first to a short and then to a long photoperiod [32]. In the latter studies it remained open whether the changes were induced by the photoperiod or whether they have an endogenous component. Our results indicate that there is an endogenous component contributing to the seasonal changes in sleep.

The stability of sleep duration seems to be a feature of animals that exhibit a polyphasic sleep-wake pattern [27-31], since humans that typically have a monophasic sleep pattern, did increase sleep duration during the winter months [22-24]. The changes in SWA in NREM sleep reflected the changes in the amount of sleep within the light or dark period. In winter, when sleep was more evenly distributed between the light and dark period, SWA showed only a minimal LD amplitude. In contrast, in summer when the hamsters were more awake during the dark period, SWA exhibited concomitantly higher values compared to the light phase. Thus, hamsters in the summer physiological state sleep less but more intensively during the dark period compared to their sleep in winter. These data are in accordance with the two-process model of sleep regulation that predicts that the homeostatic Process S, quantified by SWA reflects the previous sleep-wake history [35].

The sleep-wake redistribution between the winter and summer physiology animals was reflected also in the frequency and duration of vigilance state episodes. Waking episode duration was lower and the frequency of NREM sleep episodes was higher during the light period in summer physiology, when the hamsters slept more. No changes occurred in the hamsters in winter physiology when sleep was more evenly distributed over the 24 h.

Although day-length appears to be the primary environmental cue that the Djungarian hamster uses to initiate seasonally appropriate physiological and

behavioural changes [2, 8, 14], ambient temperature and food restriction markedly affect the photoperiodic responses [14]. In order to make sure that the changes we observed were reflecting endogenous mechanisms, several correlations were computed. Not even a trend between any of the effects on sleep and T_A or brain temperature was observed. Also in a previous study, EEG power density in the slow-wave range had increased when hamsters were recorded first in a short photoperiod followed by a long photoperiod [32]. In both studies other EEG frequencies were not affected. We have repeatedly observed a decrease of EEG power density over time in rats and hamsters chronically implanted with EEG electrodes. It is therefore unlikely that the present increase in SWA from winter to summer was due to technical artifacts.

In a previous paper we concluded that when the Djungarian hamster prepares for the harsh environment it will encounter during winter, it dissociates sleep homeostasis from the circadian clock [32]. This was based on the data that showed that sleep homeostasis remained invariable in a long and short photoperiod, but the sleep-wake pattern had changed dramatically [31, 36]. Nevertheless, it was evident that the circadian clock remained functional, because other behavioural and physiological rhythms remained in synchrony with the light-dark cycle [32, 37], or free-ran in constant darkness [38]. The present data show that when the animal's physiology returns to its summer characteristics while the short photoperiod and low T_A are maintained, the circadian clock seems to regain control over the sleep-wake behaviour. This indicates that physiological changes occurring in the hamsters as they adapt to different photoperiods encompass also an interaction between the circadian clock and sleep homeostatic mechanisms.

Little is known about the mechanisms that underlie this interaction. Among the many adaptations to the short photoperiod, the most important advantage for survival the Djungarian hamster gains from the short photoperiod physiology is the change in metabolic rate [37]. Recent developments in narcolepsy research integrate sleep and metabolism and suggest that changes in metabolic rate do influence sleep regulation [39]. Also hormonal changes may be involved in the relation between sleep homeostasis and the circadian clock. For example it has been hypothesized that melatonin can serve both as a clock and as a calendar [1, 9, 40]. In the Djungarian hamster and the European hamster the duration and amplitude of melatonin secretion depends on photoperiod e.g. [6, 11, 41], (A. Stieglitz, PhD thesis, 1995). When the duration of darkness was increased the onset of melatonin synthesis was delayed in both species, while the end corresponded to lights on in the Djungarian hamster only when adaptation to the photoperiod was sufficiently long [6, 11, 42]. Thus, in the European hamster an endogenous, seasonal component became evident in the short photoperiod, where the end of melatonin secretion was unrelated to the time of lights on. The evidence that melatonin may have a direct effect on sleep in the Djungarian hamster [43, 44] or in the rat [43] is inconclusive.

Previous comparisons of the EEG power spectra in NREM sleep have shown a frontal predominance of the low EEG frequencies in humans [45-47], rats [48, 49], mice [50], and Djungarian hamsters [51]. Interestingly, the frontal

predominance in EEG power density in NREM sleep was not affected by the seasonal changes in the animal's physiology, supporting the interpretation that frontal predominance may reflect a functional component that is related to previous waking activities.

Conclusions

Our data show that light-dark differences in sleep-wake behavior and the time-course of EEG SWA recover when the hamsters spontaneously exhibit changes related to long photoperiod physiology. The circadian clock seems to regain control of the circadian sleep-wake distribution.

Methods

Animals. Adult Djungarian hamsters (*Phodopus sungorus*) raised under a natural photoperiod in summer, were kept individually in Macrolon cages (36x20x35 cm) with food and water available ad libitum, and maintained in a short photoperiod with 8-h light - 16-h dark (LD, light from 09:00 - 17:00 h; 7 Watt OSRAM DULUX EL energy saving lamp, approximately 30 lux). Mean ambient temperature (T_A) was 15.5 ± 0.2°C.

Surgery. When the weight reduction and the fur colour index (# 5-6 on the index scale 1-6, according to Figala et al [52]) as well as the gonadal regression indicated a strong adaptation to the short photoperiod (Figure 1), the 15 best adapted hamsters of a total of 32 were selected for i.p. implantation of temperature-sensitive transmitters (model X-M, Mini-mitter). At the age of 5.3±0.4 months the hamsters (mean weight 26.6±1.3 g, n=15) were implanted under deep anaesthesia (Ketalar® 75 mg/kg, Parke-Davis; Rompun® 4 mg/kg, Bayer, i.p.) with gold-plated miniature screws (0.9 mm diameter) that served as EEG electrodes. Screws were placed epidurally over the right parietal cortex (2 mm lateral to midline and 2 mm posterior to bregma), right frontal cortex (2 mm lateral to midline and 2 mm anterior to bregma) and a reference electrode was placed over the cerebellum (2 mm posterior to lambda, on midline). A thermistor (Thermometrics, P20, R (25°C) = 1 kΩ, max. diam. = 0.5mm, accuracy ±0.05°C) was inserted horizontally between the skull and dura through a hole over the left frontal cortex (2-3 mm lateral to midline and 2 mm anterior to bregma) to measure cortical temperature (T_{CRT}). Two gold wires (diameter 0.2 mm) inserted into the neck muscles served to record the electromyogram (EMG). The electrodes and thermistor were soldered to stainless steel wires and to a plug that was fixed to the skull with dental cement [53]. Animals were connected to the cables and allowed to recover at least two weeks.

Experimental protocol. The two EEGs, EMG and T_{CRT} were continuously recorded for 24-h when the animal's physiology was in 'winter' conditions (January - February). After 'summer' physiology was manifest in all animals, i.e. fur colour changed from white-grey to brown-grey and regrowth of gonads was evident, a second 24-h record was obtained in March. The short photoperiod and low T_A were maintained throughout the entire experiment. Six hamsters contributed to both days, whereas four and five hamsters contributed with a recording in winter or summer, respectively.

Data acquisition and analysis. The EEG and EMG signals were amplified (amplification factor approx. 2,000), conditioned by analogue filters (high-pass filter: -3 dB at 0.016 Hz; low-pass filter: -3 dB at 40 Hz, less than -35 dB at 128 Hz) sampled with 512 Hz, digitally filtered (EEG: low-pass FIR filter 25 Hz; EMG: band-pass FIR filter 20-50 Hz) and stored with a resolution of 128 Hz. EEG power spectra were computed for 4-s epochs by a Fast Fourier Transform (FFT) routine. Adjacent 0.25-Hz bins were averaged into 0.5-Hz (0.25 - 5.0 Hz) and 1.0-Hz (5.25-25.0 Hz) bins. The EMG was full-wave rectified and integrated over 4-s epochs, T_{CRT} and T_A inside the cage were sampled at 4-s intervals. Before each recording the EEG and EMG channels were calibrated with a 10 Hz sine wave, 300 μV peak-to-peak signal.

The three vigilance states NREM sleep, REM sleep and waking were scored for 4-s epochs as in previous studies [30, 53]. Vigilance states were determined off-line by visual inspection of the parietal and frontal EEG and EMG records and EEG power in the slow-wave range (0.75-4.0 Hz). Epochs containing EEG artifacts in both derivations or in a single derivation were excluded from spectral analyses of both EEG derivations (14.2 $\pm$ 0.9 SEM % of recording time. Artifacts occurred mainly during active waking: 24.4 $\pm$ 1.8 SEM % of waking). Vigilance states could always be determined.

The duration and frequency of vigilance state episodes were determined according to criteria described previously [30, 53]. Differences in the EEG spectrum between the winter and summer recording were tested by ANOVA for repeated measures (rANOVA) or ANOVA. Whenever ANOVA reached significance, differences were evaluated by post hoc two-tailed paired *t*-tests within days or by unpaired *t*-test between two days. LD differences were tested with post hoc two-tailed paired *t*-tests within days or by unpaired *t*-test between two days.

All statistical comparisons of data from recordings performed in hamsters in winter and summer physiology were performed twice, once for the n=6 hamsters which had been recorded both in winter and summer physiology (paired tests), and once for the entire group (n=10-11; unpaired tests).

Authors' contribution

All authors participated in the planning and execution of the study; all authors contributed to the draft and read and approved the manuscript.

Acknowledgements

We thank Dr. G. Heldmaier for providing us with the hamsters and Dr. A. Borbély for critical reading of the manuscript. The study was supported by the Swiss National Science Foundation grant nr. 3100-053005.97/2.

References

1. TJ Bartness, BD Goldman: **Mammalian pineal melatonin: a clock for all seasons**. *Experientia* 1989, **45**:939-945.
2. K Hoffmann: **The influence of photoperiod and melatonin on testis size, body weight, and pelage colour in the Djungarian hamster (*Phodopus sungorus*)**. *J. Comp. Physiol.* 1973, **85**:267-282.

3. K Hoffmann: **Testicular involution in short photoperiods inhibited by melatonin**. *Naturwissenschaften* 1974, **61**:364-365.
4. K Hoffmann: **Effects of short photoperiods on puberty, growth and moult in the Djungarian hamster (*Phodopus sungorus*)**. *J Reprod Fertil* 1978, **54**:29-35.
5. J Arendt, AM Symons, C Laud: **Pineal function in the sheep: Evidence for a possible mechanism mediating seasonal reproductive activity**. *Experientia* 1981, **37**:584-586.
6. H Illnerova, K Hoffmann, J Vanecek: **Adjustment of pineal melatonin and N-acetyltransferase rhythms to change from long to short photoperiod in the Djungarian hamster *Phodopus sungorus***. *Neuroendocrinology* 1984, **38**:226-231.
7. L Tamarkin, CJ Baird, OF Almeida: **Melatonin: a coordinating signal for mammalian reproduction?** *Science* 1985, **227**:714-720.
8. A Lerchl, S Schlatt: **Influence of photoperiod on pineal melatonin synthesis, fur color, body weight, and reproductive function in the female Djungarian hamster, *Phodopus sungorus***. *Neuroendocrinology* 1993, **57**:359-364.
9. RJ Reiter: **The melatonin rhythm: both a clock and a calendar**. *Experientia* 1993, **49**:654-664.
10. BD Goldman: **The Siberian hamster as a model for study of the mammalian photoperiodic mechanism**. *Adv Exp Med Biol* 1999, **460**:155-164.
11. B Vivien-Roels, B Pitrosky, M Zitouni, A Malan, B Canguilhem, D Bonn, P Pevet: **Photoperiodic control of the seasonal variations in the daily pattern of melatonin synthesis in the European hamster, *Cricetus cricetus***. *Ann N Y Acad Sci* 1998, **839**:386-388.
12. JA Elliott, TJ Bartness, BD Goldman: **Role of short photoperiod and cold exposure in regulating daily torpor in Djungarian hamsters**. *J. Comp. Physiol. [A]*. 1987, **161**:245-253.
13. A Stieglitz, S Steinlechner, T Ruf, G Heldmaier: **Cold prevents the light induced inactivation of pineal N- acetyltransferase in the Djungarian hamster, *Phodopus sungorus***. *J Comp Physiol [A]* 1991, **168**:599-603.
14. T Ruf, A Stieglitz, S Steinlechner, JL Blank, G Helmaier: **Cold exposure and food restriction facilitate physiological responses to short photoperiod in Djungarian hamsters (*Phodopus sungorus*)**. *J. Exp. Zool.* 1993, **267**:104-112.
15. JE Larkin, J Jones, I Zucker: **Temperature dependence of gonadal regression in Syrian hamsters exposed to short day lengths**. *Am J Physiol Regul Integr Comp Physiol* 2002, **282**:R744-R752.
16. S Messager, AW Ross, P Barrett, PJ Morgan: **Decoding photoperiodic time through Per1 and ICER gene amplitude**. *Proc Natl Acad Sci U S A* 1999, **96**:9938-9943.
17. B Nuesslein-Hildesheim, JA O'Brien, FJ Ebling, ES Maywood, MH Hastings: **The circadian cycle of mPER clock gene products in the suprachiasmatic nucleus of the siberian hamster encodes both daily and seasonal time**. *Eur J Neurosci* 2000, **12**:2856-2864.
18. S Daan, U Albrecht, GT van der Horst, H Illnerova, T Roenneberg, TA Wehr, WJ Schwartz: **Assembling a clock for all seasons: are there M and E oscillators in the genes?** *J Biol Rhythms* 2001, **16**:105-116.
19. G Lincoln, S Messager, H Andersson, D Hazlerigg: **Temporal expression of seven clock genes in the suprachiasmatic nucleus and the pars tuberalis of the sheep: evidence for an internal coincidence timer**. *Proc Natl Acad Sci U S A* 2002, **99**:13890-13895.
20. JM Walker, EH Haskell, RJ Berger, HC Heller: **Hibernation and circannual rhythms of sleep**. *Physiol. Zool.* 1980, **53**:8-11.
21. V Barre, A Petterrousseaux: **Seasonal-Variations in Sleep-Wake Cycle in *Microcebus murinus***. *Primates* 1988, **29**:53-64.
22. A Wirz-Justice, RA Wever, J Aschoff: **Seasonality in freerunning circadian rhythms in man**. *Naturwissenschaften* 1984, **71**:316-319.
23. TA Wehr: **The durations of human melatonin secretion and sleep respond to changes in daylength (photoperiod)**. *J Clin Endocrinol Metab* 1991, **73**:1276-1280.
24. TA Wehr, DE Moul, G Barbato, HA Giesen, JA Seidel, C Barker, C Bender: **Conservation of photoperiod-responsive mechanisms in humans**. *Am. J. Physiol.* 1993, **265**:R846-R857.
25. M Kohsaka, N Fukuda, K Honma, S Honma, N Morita: **Seasonality in human sleep**. *Experientia* 1992, **48**:231-233.
26. I Tobler: **Behavioral sleep in the Asian elephant in captivity**. *Sleep* 1992, **15**:1-12.
27. AA Borbély, HU Neuhaus: **Daily pattern of sleep, motor activity and feeding in the rat: effects of regular and gradually extended photoperiods**. *J. Comp. Physiol. [A]*. 1978, **124**:1-14.

28. DJ Dijk, S Daan: **Sleep EEG spectral analysis in a diurnal rodent: *Eutamias sibiricus***. *J Comp Physiol [A]* 1989, **165**:205-215.
29. P Franken, I Tobler, AA Borbély: **Varying Photoperiod in the Laboratory Rat - Profound Effect on 24-H Sleep Pattern but No Effect on Sleep Homeostasis**. *Am J Physiol Regul Integr Comp Physiol* 1995, **38**:R691-R701.
30. T Deboer, I Tobler: **Shortening of the photoperiod affects sleep distribution, EEG and cortical temperature in the Djungarian hamster**. *J. Comp. Physiol. [A]*. 1996, **179**:483-492.
31. T Deboer, I Tobler: **Vigilance state episodes and cortical temperature in the Djungarian hamster: the influence of photoperiod and ambient temperature**. *Pflugers Arch.* 1997, **433**:230-237.
32. T Deboer, VV Vyazovskiy, I Tobler: **Long photoperiod restores the 24-h rhythm of sleep and EEG slow-wave activity in the Djungarian hamster (*Phodopus sungorus*)**. *J Biol Rhythms* 2000, **15**:429-436.
33. RJ Reiter: **Evidence for refractoriness of the pituitary-gonadal axis to the pineal gland in golden hamsters and its possible implications in annual reproductive rhythms**. *Anat Rec* 1972, **173**:365-371.
34. AS Loudon, N Ihara, M Menaker: **Effects of a circadian mutation on seasonality in Syrian hamsters (*Mesocricetus auratus*)**. *Proc R Soc Lond B Biol Sci* 1998, **265**:517-521.
35. AA Borbély: **A two process model of sleep regulation**. *Hum. Neurobiol.* 1982, **1**:195-204.
36. T Deboer, I Tobler: **Slow waves in the sleep electroencephalogram after daily torpor are homeostatically regulated**. *Neuroreport* 2000, **11**:881-885.
37. G Heldmaier, S Steinlechner: **Seasonal pattern and energetics of short daily torpor in the Djungarian hamster, *Phodopus sungorus***. *Oecologia* 1981, **48**:265-270.
38. T Ruf, S Steinlechner, G Heldmaier: **Rhythmicity of body temperature and torpor in the Djungarian hamster, *Phodopus sungorus***. In: *Living in the cold II* Edited by A Malan, B Canguilhem. pp. 53-61: John Libbey Eurotext Ltd.; 1989: 53-61.
39. S Taheri, JM Zeitzer, E Mignot: **The role of hypocretins (orexins) in sleep regulation and narcolepsy**. *Annu Rev Neurosci* 2002, **25**:283-313.
40. B Pitrosky, R Kirsch, B Vivien-Roels, I Georg-Bentz, B Canguilhem, P Pevet: **The photoperiodic response in Syrian hamster depends upon a melatonin- driven circadian rhythm of sensitivity to melatonin**. *J Neuroendocrinol* 1995, **7**:889-895.
41. ML Garidou, B Vivien-Roels, P Pevet, J Miguez, V Simonneaux: **Mechanisms regulating the marked seasonal variation in melatonin synthesis in the European hamster pineal gland**. *Am J Physiol Regul Integr Comp Physiol* 2003, **284**:R1043-R1052.
42. K Hoffmann, H Illnerova, J Vanecek: **Comparison of pineal melatonin rhythms in young adult and old Djungarian hamsters (*Phodopus sungorus*) under long and short photoperiods**. *Neurosci Lett* 1985, **56**:39-43.
43. R Huber, T Deboer, B Schwierin, I Tobler: **Effect of melatonin on sleep and brain temperature in the Djungarian hamster and the rat**. *Physiol. Behav.* 1998, **65**:77-82.
44. T Deboer, I Tobler: **Chronic administration of melatonin reduces REM sleep in the Djungarian hamster (*Phodopus sungorus*)**. *Neurosci Lett* 1997, **231**:118-122.
45. E Werth, P Achermann, AA Borbély: **Brain topography of the human sleep EEG: antero-posterior shifts of spectral power**. *Neuroreport* 1996, **8**:123-127.
46. C Cajochen, R Foy, DJ Dijk: **Frontal predominance of a relative increase in sleep delta and theta EEG activity after sleep loss in humans**. *Sleep Res Online* 1999, **2**:65-69.
47. LA Finelli, AA Borbély, P Achermann: **Functional topography of the human nonREM sleep electroencephalogram**. *Eur J Neurosci* 2001, **13**:2282-2290.
48. B Schwierin, P Achermann, T Deboer, A Oleksenko, AA Borbély, I Tobler: **Regional differences in the dynamics of the cortical EEG in the rat after sleep deprivation**. *Clinical Neurophysiology* 1999, **110**:869-875.
49. VV Vyazovskiy, AA Borbély, I Tobler: **Interhemispheric sleep EEG asymmetry in the rat is enhanced by sleep deprivation**. *Journal of Neurophysiology* 2002, **88**:2280-2286.
50. R Huber, T Deboer, I Tobler: **Topography of EEG dynamics after sleep deprivation in mice**. *J Neurophysiol* 2000, **84**:1888-93.
51. S Palchykova, T Deboer, I Tobler: **Selective sleep deprivation after daily torpor in the Djungarian hamster**. *J Sleep Res* 2002, **11**:313-319.
52. J Figala, K Hoffmann, G Goldau: **Zur Jahresperiodik beim Dsungarischen Zwerghamster Phodopus sungorus Pallas**. *Oecologia* 1973, **12**:89-118.

53. T Deboer, P Franken, I Tobler: **Sleep and cortical temperature in the Djungarian hamster under baseline conditions and after sleep deprivation**. *J. Comp. Physiol. [A]*. 1994, **174**:145-155.

Paper 3

Sleep deprivation and daily torpor impair object recognition in Djungarian hamsters

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Published in Physiology & Behavior 2006: 87(1), 144-153

PALCHYKOVA, S., CRESTANI, F., MEERLO, P., TOBLER, I. Sleep deprivation and daily torpor impair object recognition in Djungarian hamsters. PHYSIOL BEHAV 56(6) 000-000, 2005. - Sleep has been shown to play a facilitating role in memory consolidation, whereas sleep deprivation leads to performance impairment both in humans and rodents. The effects of 4-hr sleep deprivation on recognition memory were investigated in the Djungarian hamster (*Phodopus sungorus*). Because sleep during the first hours after daily torpor has many similarities to recovery from sleep deprivation, the effects of spontaneous torpor on object recognition were also assessed.

A 4-hr sleep deprivation, starting immediately after an object learning task, diminished the ability of the hamsters to: 1. discriminate between an already encountered object (target) and a novel object presented in a novel context, 2. retrieve a target within a complex spatial scene and 3. detect a spatial rearrangement of familiar objects in a familiar context. Plasma stress hormone levels were similar in sleep-deprived and control hamsters. The occurrence of a daily torpor episode during retention was associated with impaired old-new object discrimination performance in the more effortful complex spatial scene task only, and in a two-object choice situation in a novel context no torpor-induced deficit was found.

Our results show that post learning sleep deprivation and daily torpor induce a deficit in familiar object retrieval performance in a complex spatial scene, while sparing familiarity-based recognition and novelty processing. Sleep deprivation during the first 4 hrs of memory consolidation hampered also recency memory for discrete objects. Stress was not a factor contributing to the sleep deprivation-induced impairment.

Keywords: Djungarian hamster, daily torpor, sleep deprivation, recognition memory, stress, hormones, ACTH, corticosterone, cortisol, behavior

1. Introduction

There is a major renewal of interest in the relationship between sleep and memory. Significant advances have been made in the understanding of the molecular and physiological mechanisms underlying these brain functions. Various approaches have been used to study the changes in sleep after learning, and the consequences of sleep deprivation on the formation, expression and retrieval of memories (Maquet et al., 2000, Huber et al., 2004, Walker and Stickgold, 2004). In humans sleep seems to play a facilitatory role on the consolidation of procedural and declarative memories (Karni et al., 1994, Smith, 1995, Stickgold, 1998, Gais et al., 2000), whereas sleep deprivation leads to their impairment (Harrison and Horne, 2000, Mu et al., 2005). In rodents, REM sleep is increased after task learning (Hennevin and Hars, 1987, Datta, 2000, Ambrosini and Giuditta, 2001, Sanford et al., 2003, Datta et al., 2004), and sleep deprivation leads to impairment of various forms of memory (e.g., (Smith and Rose, 1996, Smith et al., 1998, Youngblood et al., 1999, Graves et al., 2003, McDermott et al., 2003)). However, because of methodological differences, potential confounding effects of stress, and controversial evidence, the fundamental relevance of sleep in memory processing has been questioned (Vertes, 2004).

We investigated the relationship between sleep and memory processes underlying recognition of objects in the Djungarian hamster. Four hr sleep deprivation in this photoperiodic species leads to an increase in slow-wave activity (SWA) in non-rapid eye-movement (NREM) sleep (Deboer et al., 1994, Deboer and Tobler, 2000). This effect is observed independently of whether sleep deprivation is performed during summer or winter (Deboer and Tobler, 1994, Deboer and Tobler, 2000, Palchykova et al., 2002, Deboer and Tobler, 2003). SWA in NREM sleep is a reliable predictor of sleep intensity (Achermann and Borbély, 2003, Vyazovskiy and Tobler, 2005) that increases proportionally to the duration of previous wakefulness and gradually declines during sleep. Under winter photoperiod (LD 8 h light : 16 h dark) the Djungarian hamster spontaneously exhibits daily torpor, a hypometabolic state lasting several hours (Heldmaier and Ruf, 1992). The remarkable post-torpor increase of SWA in NREM sleep closely resembled recovery after sleep deprivation leading to the hypothesis that also during daily torpor a sleep deficit is accumulated (Deboer and Tobler, 1994, Deboer and Tobler, 2000, Deboer and Tobler, 2003). In hamsters approximately 6 hrs of torpor and 4 hrs of sleep deprivation induced a comparable increase in SWA (Deboer and Tobler, 2003) attaining baseline levels within 3 or 6 hrs of recovery (Deboer et al., 1994, Palchykova et al., 2002). Hibernation, another hypothermic condition resembling sleep deprivation (Daan et al., 1991, Trachsel et al., 1991), diminished performance of ground squirrels in conditioning tasks (Millesi et al., 2001).

Recognition is a universal ability to remember, i.e. to judge that something has occurred previously (Dudai, 2004b). It is based on the capacity to detect familiarity or recency, and to discriminate and recollect specific features of objects, persons or events previously encountered in the same or in a different situational context (Brown and Aggleton, 2001). Familiarity-based recognition of objects is based on the perception that an item has been

encountered previously ("familiarity *vs* novelty"). Rats and mice explore the familiar object less and show more interest when they encounter something new (Ennaceur and Delacour, 1988, Steckler et al., 1998). Recency-based recognition refers to memory of the temporal order of item presentation ("old *vs* new"). Three one-trial discrimination learning tasks with different degree of difficulty of stimulus discrimination, were designed to examine the effects of sleep deprivation performed during the early memory consolidation phase and of spontaneous daily torpor on memory retrieval for discrete objects. Because emotional arousal can influence performance, and sleep deprivation may be stressful, plasma levels of pituitary and adrenal stress hormones were measured in control hamsters subjected to the learning task only and compared with hamsters subjected to the learning task and to sleep deprivation.

2. Methods

2.1. Animals

The Cantonal Veterinary Office of Zurich approved all experimental procedures. Adult Djungarian hamsters (*Phodopus sungorus*; *n*=114, 65 males and 49 females), weighing 36 ± 7 g (SD), were kept individually in Macrolon cages (36 x 20 x 35 cm) with food and water available *ad libitum*. The Djungarian hamster is a photoperiodic rodent (Goldman, 1999), therefore all experiments were performed during the winter months. The animals were raised in a natural photoperiod during summer, and then acclimated to winter conditions with an 8:16 hr light:dark cycle (light onset at 9 A.M.; approximately 30 lux) and 14 °C ambient temperature.

As soon as the hamsters commenced to adapt to the winter photoperiod, as indicated by body weight reduction (33 ± 7 g) and a change in fur color towards white (index at surgery 3.1 ± 0.1; scale ranging from 1 to 6; (Figala et al., 1973)), they were implanted intraperitoneally (i.p.) with temperature-sensitive transmitters (model X-M, Mini-mitter) under deep anaesthesia (Ketamine, KETALAR® 75 mg/kg, Parke-Davis; Xylasine, ROMPUN® 4 mg/kg, Bayer, i.p.). At least four weeks were allowed for recovery. Behavioral testing was performed in a temperature-controlled chamber under dim red lighting conditions (< 5 lux) during the dark period at the age of 6 to 10 months. Individuals were exposed to one of three behavioural tasks.

2.2. Behavioral tasks

Three learning tasks were adapted from the rat object recognition memory test, which is based on the spontaneous tendency of rodents to explore novel objects more than familiar ones (Ennaceur and Delacour, 1988). The tasks comprised a study phase, delay phase and test phase. Before the study phase, all hamsters were familiarized with the experimental context on one or two consecutive days. They were placed individually in the empty enclosure for 10 min. The floor of the enclosures was covered with soiled wood chippings collected from cages of several hamsters, in order to saturate it with odors of conspecifics. Familiarization, study and test phases were performed

at the same time of day and kept constant for individual hamsters. In the study phase, the animals were exposed for 5 min to a sample stimulus, consisting of objects. After a delay phase of 24 hrs, in the test phase, the hamsters were exposed for 5 min simultaneously to an object previously encountered in the study phase (target item) and new choice items. Nine sets of 3 or 4 identical objects (16 to 18 cm^3) differing in shape, color and texture and with no biological relevance were used. The objects and their spatial location were randomised among the animals. Video recordings were obtained throughout the experimental phases.

The first behavioral task tested memory for a discrete object. The study phase was performed in a T-shaped enclosure (grey plastic, 25 cm high), comprising three distinct compartments (10 cm x 10 cm) individualized by three removable partitions and a central zone (20 cm x 10 cm). In the course of the experiment, the location and orientation of the enclosure within the test room were kept constant, thus defining a spatial attribute to three compartments (West, East and South). After two familiarization days, the hamsters were exposed to a single novel object ("novel" because it was never seen before; served as target) located in the middle of the West compartment during the study phase. To minimise the use of spatial and contextual cues to retrieve object information, the test phase was run in a novel rectangular enclosure (30 x 20 x 20 cm) subdivided into 6 inter-connected compartments (10 x 10 cm). A duplicate of the target object (familiar or old) was placed in the middle of the Northwest compartment and presented together with another "novel" object placed in the Northeast compartment. The animals were introduced into the enclosure by the Southwest compartment.

The second task tested memory for a complex scene by exposing a new group of hamsters to a triplet of objects in the T-enclosure, which served as the spatial context for both the study and the test phase. In the study phase, a different novel object was placed in the middle of each of the three compartments. In the test phase, the hamsters were exposed to a new triplet of objects consisting of an identical copy of the original object (target) located in the West compartment and two other different novel objects.

The third task tested memory for the spatial location of an object using a square enclosure (75 x 75 x 37 cm) throughout the experiment. After a single 10-min familiarization session, during the study phase hamsters were allowed to investigate two identical copies of a novel object placed in two adjacent corners. In the test phase, two new identical copies of the object were used: one was placed in its original location (target) and the other into another corner (choice copy). The position of the target was counterbalanced among the animals.

2.3. Sleep deprivation

Hamsters were subjected to sleep deprivation by "gentle handling" (Deboer et al., 1994, Tobler et al., 1997a) for 4 hrs immediately following the study phase, thereby interfering with the early phase of memory consolidation. The effect of this duration of sleep deprivation on recovery sleep is comparable to a 6-h sleep deprivation in mice (Tobler et al., 1996a, Huber et al., 2000b). Briefly, the animals were continuously observed, and when they assumed a sleep posture, they were disturbed by introducing tissues into the cage or by

mild acoustic stimulation (tapping on the cage). Special care was taken not to interfere with feeding and drinking behavior. Control hamsters were returned to their home cage immediately after study and were left undisturbed.

2.4. Daily torpor

To identify and verify the regular occurrence of torpor episodes, body temperature was continuously recorded for several days at 5-min intervals. Torpor usually occurred after light onset, the rest phase of this species, and approximately at the same time of day within individuals. The beginning or the end of a torpor episode was defined as body temperature below or above 32°C, respectively (Ruf et al., 1989, Deboer and Tobler, 1994). Behavioral testing started 1 - 7.5 hrs after dark onset, at least 3 hrs after the end of torpor, once the hamsters exhibited at least two torpor episodes per week.

Despite limiting the timing of the interventions to the beginning of the dark period and their short duration (< 10 min), only 22 of 72 hamsters exhibited torpor spontaneously the day after the study phase. They were assigned to the “torpor groups”. The torpor episodes occurred 8 – 17 hrs after the study phase and lasted between 4 – 17 hrs (6.4 ± 0.4 hrs, n = 22). The remaining animals, whose body temperature did not decrease below 32°C between the study and test phase, served as controls.

2.5. Plasma ACTH, cortisol and corticosterone assays

Hamsters were placed individually in a T-enclosure 10 weeks after the test in a square enclosure (n = 32). They were exposed to three different novel objects for 5 min and killed by decapitation for blood collection 30 min (n = 8) or 4 hrs later (n = 7). Two groups were subjected to 30 min or 4 hrs sleep deprivation, starting immediately after exposure to novelty, and were killed thereafter (n = 8 per group). An undisturbed control group (n = 8) was kept in individual home-cages and killed at the same time of day as the animals used for the 30-min time point. Trunk blood was collected in K2E EDTA K2 tubes (Vacuette® 2 ml, Greiner Bio-One Vacuette Schweiz GmbH) at 0 °C, centrifuged at 2600 g at 4 °C for 15 min. The supernatant was stored at -80 °C in cryotubes for later analysis. Plasma ACTH, cortisol and corticosterone concentrations were determined by radioimmunoassay (ACTH kit, Nichols Institute Diagnostics, Bad Vilbel, Germany; cortisol kit, Diagnostic Systems Laboratories, Webster, USA; corticosterone kit, MP Biomedicals, Costa Mesa, USA).

2.6. Variables and statistics

Locomotor activity and interaction with objects were quantified by visual off-line scoring of the tapes. Locomotion was defined as the number of crossings of 4 virtual lines in the T-enclosure or of 6 compartments of the rectangular enclosure. The exploratory behavior towards objects was quantified by counting the number of investigations (approach of the nose to a distance < 2 cm and/or contact with the objects) and their duration. An object exploration ratio, i.e. the difference in exploration time of the target and novel object(s) divided by the total time spent exploring all objects, was calculated. A ratio > 0

for 2 objects (or 0.33 for 3 objects) indicated a greater exploration of the novel object(s) and a ratio < 0 (or 0.33), a greater exploration of the target.

Behavioral data were analysed by ANOVA (unweighted means or hierarchical solution depending on group size) with group (control vs sleep deprivation or control vs torpor) as between subject factor and object or phase (study vs test) as within subject factor. Post-hoc Tukey's pair-wise comparisons were performed whenever appropriate. For the analysis of plasma hormone levels a two-way ANOVA with group and time as between subject factors was used. Whenever the Levene's test for homogeneity of variance was significant, data were subjected to decimal logarithm transformation. The normality of distribution was assessed with the procedure Univariate (SAS) that performs the Kolmogorov-Smirnov, Shapiro-Wilk, Anderson-Darling or Cramer-von Mises tests depending on the sample size. Unpaired or paired t-tests were used for between and within group comparisons, respectively. Non-parametric Kruskal-Wallis and Wilcoxon signed-rank tests were used when the sample sizes were equalled six or if data were not normally distributed. Results were expressed as mean ± SE.

3. Results

The object recognition memory test was developed for rats, mice and humans. Its adaptation for hamsters showed that they approach the objects to the same extent as 129/SvJ mice tested in a similar paradigm, and stay twice longer investigating them (Table 1).

3.1. Effects of sleep deprivation on recognition memory

3.1.1. Effects of sleep deprivation on memory for discrete objects

The impact of sleep deprivation on familiarity and recency-based memories for a discrete object (target) encountered previously in a familiar T-enclosure was examined in a simple two-object choice situation in a novel context (rectangular enclosure).

Table 1. Exploratory behavior towards novel objects

Objects	Djungarian hamsters (*n* = 55)		129/SvJ mice (*n* = 26)	
	Number	Duration (s)	Number	Duration (s)
O1	12.8 ± 0.5	40.1 ± 1.9	16.9 ± 0.8	20.0 ± 1.5
O2	12.9 ± 0.7	37.0 ± 1.7	15.7 ± 0.9	18.4 ± 1.3
O3	11.3 ± 0.7	35.8 ± 2.1	17.3 ± 0.8	22.4 ± 2.0

Spontaneous exploratory behavior towards novel objects presented in a familiar T-enclosure. Mean values (± SE) of the number of investigations and their duration (in seconds) for three novel objects (O1, O2 and O3) during a 5-min study phase. The exploratory behavior of adult 129/SvJ mice tested in very similar conditions is shown for comparison (L. Prut and F. Crestani, unpublished).

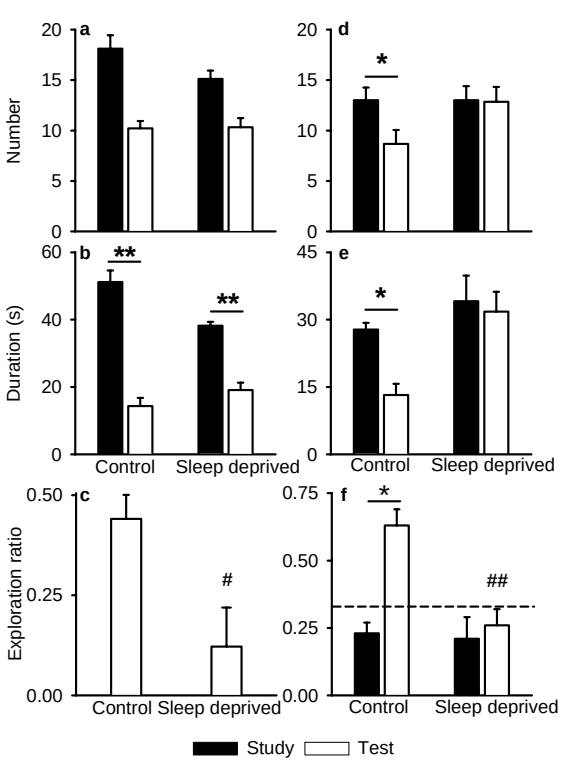

Figure 1. Effects of 4-hrs sleep deprivation on the exploratory behavior towards a target object encountered 24 hrs earlier at study. The test consisted of a simple two-object choice task in a novel context (a, b and c) or a new complex scene in a familiar context (d, e and f). a, The number of investigations of the target decreased from study to test [phase, F(1, 25) = 36.28, P < 0.001] similarly in controls (n = 18) and hamsters, which were sleep deprived by "gentle handling" immediately after study (Sleep depr.; n = 9). b, Likewise, the duration of target investigations was decreased at test in both groups [phase, F(1, 25) = 87.57, P < 0.001]. c, At test, the exploration ratio was reduced in sleep-deprived hamsters compared to controls. d, Sleep-deprived hamsters (n = 6) failed to decrease the number of target investigations at test in contrast to controls (n = 6). e, The duration of target investigations decreased from study to test in controls but not in sleep-deprived animals. f, At test, sleep-deprived hamsters did not differentiate between the target and the two new novel objects, in contrast to control animals, which showed a clear preference for novel objects, as indicated by the increase in the exploration ratio from study to test. Mean values ± SE. * $p < 0.05$ and ** $p < 0.01$ versus study, Wilcoxon and Tukey, respectively; $^{\#}$ $p < 0.05$ and $^{\#\#}$ $p < 0.01$ versus control, unpaired *t*-test and Kruskal-Wallis, respectively. The dashed line represents the chance level (0.33).

Exploratory behavior towards the target revealed a significant phase effect on the number of investigations [$P < 0.001$] and their duration [$P < 0.001$, after logarithmic transformation] (Figs. 1a and b). The overall group effect was not significant for either variable [number, $F_{(1, 25)} = 1.05$; duration, $F_{(1, 25)} = 0.12$]. Interaction was significant for duration [$F_{(1, 25)} = 6.60$, $P < 0.02$] but not for number [$F_{(1, 25)} = 2.19$]. As expected, the duration of target exploration decreased from study to test ($P < 0.01$, Tukey), indicating familiarity detection, and was similar in both groups (Figs. 1b). Moreover, at study and test the control and sleep-deprived animals did not differ (Fig. 1b).

At test the exploration ratio of sleep-deprived hamsters differed from controls [$t_{(11.73)} = 2.21$, $P < 0.05$] (Fig. 1c). Although both groups explored preferentially the novel object (mean exploration ratio elevated above chance), the sleep-deprived animals displayed a lower level of discrimination, suggesting an impaired recency-based recognition of the target.

A further analysis examined whether the animals could detect object novelty, i.e. a novel object presented in a novel context. The novel object exploration decreased from study to test [number, $F_{(1, 25)} = 24.79$ and duration, $F_{(1, 25)} = 17.90$, $P < 0.001$] and was similar in the two groups [number, $F_{(1, 25)} = 0.65$ and duration, $F_{(1, 25)} = 0.07$] (data not shown). Furthermore, irrespective of the group, the number of investigations of the two objects at test (22 ± 1, $n = 27$) was significantly higher than that of one object at study (18 ± 1, $n = 27$)

[$F_{(1, 25)}$ = 13.48, P < 0.001], but their duration was unchanged from study to test (47 ± 3 s and 49 ± 3 s, respectively, n = 27) [$F_{(1, 25)}$ = 0.99].

In summary, sleep-deprived hamsters processed the target as familiar, and detected object novelty in the novel context to the same extent as controls, but their old-new object discrimination performance was significantly less accurate, suggesting an altered recency memory.

3.1.2. Effects of sleep deprivation on memory for a complex spatial scene

The consequences of the 4 hr sleep deprivation on retrieval and discrimination performance for a specific object, presented within a complex scene, consisting of a triplet of objects spatially arranged in a familiar T-enclosure were evaluated. Already at study, the objects located in the West or East compartment were explored to the same extent, whereas those in the South compartment were approached and investigated less (P < 0.05, Tukey after ANOVA object: number, $F_{(2, 20)}$ = 8.01, P < 0.018 and duration, $F_{(2, 20)}$ = 5.95, P < 0.035). However, there was no effect of group [number, $F_{(1, 10)}$ = 2.87; duration, $F_{(1, 10)}$ = 0.76] or group x object interaction [number, $F_{(2, 20)}$ = 2.14; duration, $F_{(2, 20)}$ = 0.30]. Despite the differences at study, the exploration of the two novel objects did not differ between study and test, and was comparable in the two groups (data not shown).

Familiarity-based recognition in this complex scene was evaluated by comparing the exploratory behavior towards the target object at study and test. The two groups differed significantly in the duration of target investigations ($F_{(1, 10)}$ = 19.39, P < 0.001). In contrast to controls (P < 0.05, Wilcoxon signed-ranks test), in sleep-deprived hamsters the mean number and duration of target investigations failed to decrease from study to test (Figs. 1d and e).

In addition, sleep-deprived hamsters differed from controls in their old-new object discrimination performance. The mean exploration ratios of the two groups differed significantly at test [$H_{(1)}$ = 8.31, P < 0.004], but not at study [$H_{(1)}$ = 0.23]. At test control hamsters explored preferentially the two novel objects (P < 0.05), whereas sleep-deprived animals showed no preference (Fig. 1f). The exploration ratio increased significantly from study to test in control animals only (P < 0.05, Wilcoxon).

Thus, sleep-deprived hamsters were unable to discriminate the object encountered previously when it was presented again, but within a new complex scene after a 24-hr delay.

3.1.3. Memory for object spatial location in sleep-deprived hamsters

The effects of sleep deprivation on the formation of object location memory were assessed by subjecting control and sleep-deprived hamsters at test to a spatial rearrangement of two objects that had been encountered previously in a familiar square enclosure. At study, both groups explored the two copies of the novel object to the same extent [number, group: $F_{(1, 19)}$ = 1.10, object: $F_{(1, 19)}$ = 0.15, group x object: $F_{(1, 19)}$ = 0.01; duration, group: $F_{(1, 19)}$ = 1.28, object: $F_{(1, 19)}$ = 0.07, group x object: $F_{(1, 19)}$ = 1.69] (data not shown).

Familiarity processing was evaluated by comparing the exploratory behavior toward the object placed at the target location at study and at test. The mean number and duration of investigations decreased significantly from study to test [P = 0.05 and P < 0.001, respectively] in the two groups [group:

number, $F_{(1, 19)}$ = 0.69, duration, $F_{(1, 19)}$ = 1.63; group x phase interaction: number, $F_{(1, 19)}$ = 0.58, duration, $F_{(1, 19)}$ = 3.81], indicating intact familiarity detection of the location (Figs. 2a and b).

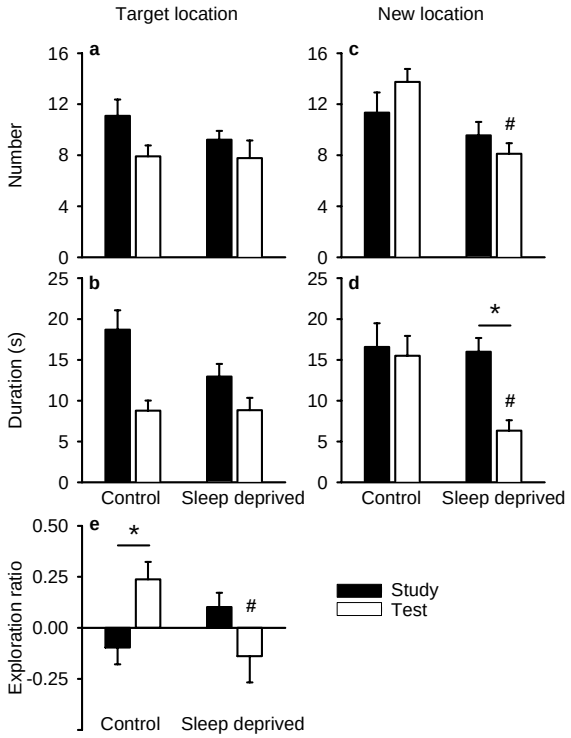

Figure 2. Influence of 4-hrs sleep deprivation on the changes in object exploration elicited by a new arrangement of a spatial scene consisting of two identical objects (Sleep depr.; *n* = 9 and their respective controls *n* = 12). *a,* The number of investigations of the target object, placed in the same spatial location as during the study phase, was lower at test in both groups [phase, $F_{(1, 19)}$ = 4.17, P = 0.05]. *b*, The duration of investigations of the target also decreased from study to test in both groups [phase, $F_{(1, 19)}$ = 21.40, P < 0.001]. *c*, Sleep-deprived hamsters approached the object transposed to a new spatial location at test less often than controls. *d*, The duration of investigations toward this object did not vary from study to test in control animals, whereas it was significantly decreased at test in sleep-deprived hamsters. *e*, At test, sleep-deprived hamsters did not show the preference towards the object positioned at the new location manifested by the control hamsters. Mean values ± SE. # *p* < 0.05 versus control, Tukey; * *p* < 0.05 versus study, Tukey or Wilcoxon.

At test sleep-deprived hamsters approached and investigated the object positioned at a new location significantly less than the controls (*P* < 0.05, Tukey after group x phase interaction: number, $F_{(1, 19)}$ = 4.57, *P* < 0.046 and duration, $F_{(1, 19)}$ = 5.92, *P* < 0.025) (Figs. 2c and d) due to a phase-dependent decrease in the mean duration of investigations of this object in sleep-deprived hamsters only (*P* < 0.05) (Fig. 2d). Moreover, the old-new object location discrimination differed between the two groups [group x phase interaction: $F_{(1, 19)}$ = 9.19, *P* < 0.007; group: $F_{(1, 19)}$ = 0.78 and phase: $F_{(1, 19)}$ = 0.24]. At test, in contrast to sleep-deprived animals, control hamsters preferentially explored the object associated with the new spatial location (*P* < 0.05); at study there was no difference between the groups (Fig. 2e). The total exploration of the objects decreased from study to test (32.3 ± 2.8 s and 20.2 ± 2.1 s, respectively, *n* = 21) [$F_{(1, 19)}$ = 33.68, *P* < 0.001] irrespective of the group [$F_{(1, 19)}$ = 0.45], indicating intact familiarity-based memory of the objects.

In summary, sleep-deprived hamsters processed the two objects as familiar, but in contrast to controls, they failed to distinguish them on the basis of their spatial location.

3.1.4. Plasma stress hormones, novelty and sleep deprivation

Plasma levels of ACTH, cortisol and corticosterone were measured in hamsters subjected to object and contextual novelty followed or not by a 30-min or 4-hr sleep deprivation. Hamsters exposed to novelty showed comparable plasma levels of ACTH, cortisol and corticosterone as

undisturbed animals which remained in their home-cage [$H_{(1)}$ = 0.40, $H_{(1)}$ = 0.00 and $H_{(1)}$ = 1.13, respectively] (Table 2). An additional 30 min or 4 hrs sleep deprivation did not increase the hormone levels significantly (Table 2) [ACTH: group, $F_{(1, 27)}$ = 0.02, group x time, $F_{(1, 27)}$ = 0.25; cortisol: group, $F_{(1, 27)}$ = 2.34, group x time, $F_{(1, 27)}$ = 1.09 and corticosterone: group, $F_{(1, 27)}$ = 1.11, group x time, $F_{(1, 27)}$ = 1.39; after logarithmic transformation].

Table 2. Effect of sleep deprivation and novelty exposure on levels of stress hormones

	Novelty		Novelty + Sleep deprivation		Baseline
	30 min	4 hrs	30 min	4 hrs	30 min
ACTH (pg/ml)	372.1 ± 143.0	467.3 ± 200.3	625.5 ± 271.4	665.1 ± 265.6	285.8 ± 88.0
Cortisol (µg/dl)	3.2 ± 0.3	2.6 ± 0.4	2.3 ± 0.3	2.5 ± 0.4	4.7 ± 1.5
Corticosterone (µg/dl)	0.5 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1

Absence of effect of novelty and sleep deprivation on plasma ACTH, cortisol and corticosterone levels. Hamsters were exposed to a novel enclosure containing three different novel objects for 5 min. Blood samples were collected 30 min (n = 8) or 4 hrs (n = 7) later. Additional two groups (n = 8) were subjected to 30 min or 4 hrs sleep deprivation after novelty exposure. Hamsters, which provided the baseline hormonal levels, were left undisturbed in their home-cages (n = 8). Mean values ± SE.

3.2. Effects of daily torpor on recognition memory

In a separate series of experiments run in parallel with the sleep deprivation experiments, we tested whether spontaneous bouts of daily torpor would affect object recognition memory.

3.2.1. Effects of daily torpor on memory for discrete objects

The consequences of a post-learning torpor episode on familiarity- and recency-based object recognition were assessed in a simple two-object choice task. The mean number and duration of target investigations decreased significantly from study to test [P < 0.001] (Figs. 3a and b), indicating a clear familiarity detection, and did not differ between the torpor and control group [number, $F_{(1, 27)}$ = 0.68; duration, $F_{(1, 27)}$ = 0.20].

At test, control and torpor hamsters displayed similar mean exploration ratios [$t_{(15.9)}$ = 0.38; Fig. 3c]. Both groups displayed a marked bias for exploring the novel object, as expected for intact recency-based memory.

The novel object presented at study elicited more exploration than the novel object presented at test [number, $F_{(1, 27)}$ = 32.67, P < 0.001 and duration, $F_{(1, 27)}$ = 8.78, P < 0.006], but this effect was similar in the two groups [group: number, $F_{(1, 27)}$ = 0.30, duration, $F_{(1, 27)}$ = 1.01; group x phase interaction: number, $F_{(1, 27)}$ = 0.00, duration, $F_{(1, 27)}$ = 0.07] (data not shown). The difference in the number of items between study (one) and test (two) had no effect on the overall amount of object exploration. Thus, the number of

object investigations increased significantly from study to test (18 ± 1 versus 22 ± 1, *n* = 29) [$F_{(1, 27)}$ = 9.63, *P* < 0.005], but their duration was stable (49.1 ± 2.7 s versus 49.4 ± 3.3 s, *n* = 29) [$F_{(1, 27)}$ = 0.00; group and group x phase interaction on both variables: *n.s.*]. Therefore, control and torpor hamsters explored the objects in the novel context to the same extent as the original target in the familiar context, suggesting that motivation was unaltered from study to test. In addition, the novelty of the context did not affect object processing in either group.

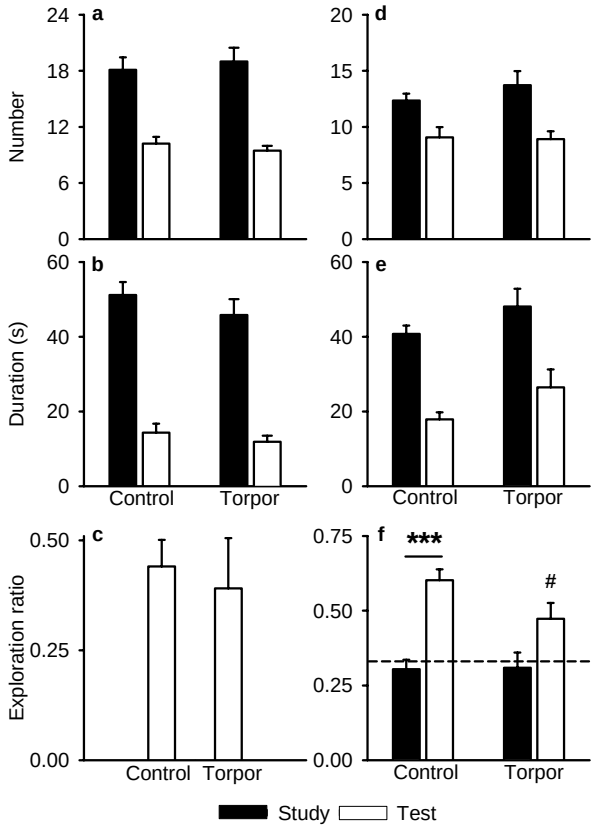

Figure 3. Influence of torpor on the exploratory behavior toward a target object encountered 24 hrs earlier at study. The test consisted of a simple two-object choice task in a novel context (*a*, *b* and *c*) or a new complex scene in a familiar context (*d*, *e* and *f*). *a,* In both control (*n* = 18) and torpor hamsters (*n* = 11), the number of investigations of the target decreased from study to test [phase, $F_{(1, 27)}$ = 75.47, P < 0.001]. *b*, The duration of target investigations was also diminished at test in the two groups [phase, $F_{(1, 27)}$ = 119.67, P < 0.001]. *c*, Control and torpor hamsters displayed a similar preference for the novel object at test, as indicated by the exploration ratios above chance. *d*, Control (*n* = 32) and torpor hamsters (*n* = 11) displayed a comparably decreased number of target investigations at test [phase, $F_{(1, 41)}$ = 20.01, P < 0.001], when presented within a new spatial scene. *e*, Likewise the duration of target investigations was decreased similarly from study to test in the two groups [phase, $F_{(1, 41)}$ = 40.24, P < 0.001]. *f*, Although the exploration ratio increased above chance from study to test in both groups $F_{(1, 41)}$ = 20.45, *P* < 0.001, torpor hamsters performed less well at test compared to controls. The difference in ratios between study and test achieved significance in controls only. Mean values ± SE. # *p* < 0.05 versus control, Kruskal-Wallis; *** *p* < 0.001 versus study, *t*-test. The dashed line represents the chance level (0.33).

The levels of locomotion were similar both in the familiar (control: 48.3 ± 3.0, *n* = 18; torpor: 45.5 ± 3.5, *n* = 11; $t_{(23.08)}$ = 0.62) and the novel context (control: 54.2 ± 2.2; torpor: 53.7 ± 4.1; $t_{(15.78)}$ = 0.11).

Therefore, familiarity- and recency-based memory for a discrete object, as well as novelty processing, were unaltered by a post learning daily torpor experience in a simple two-object choice situation in a novel context.

3.2.2. Effects of daily torpor on memory for a complex spatial scene

The capacity of torpor hamsters to retrieve a discrete object already encountered at study, when it was presented within a new complex scene in the familiar T-enclosure was investigated. Already at study, torpor hamsters differed from the controls approaching the objects in the East compartment significantly more often (16.5 ± 2.0, *n* = 11) than the controls (11.2 ± 0.8, *n* =

32; $P < 0.05$, Tukey after group x object interaction: $F_{(2, 82)} = 4.26$, $P < 0.05$), reflecting a spatial bias rather than an object preference due to the object randomisation. No difference occurred between the two other objects (data not shown). Torpor hamsters investigated the objects significantly longer than controls (mean value per object in torpor group: 46.0 ± 2.4 s; controls: 39.1 ± 1.3 s; $F_{(1, 41)} = 7.70$, $P < 0.01$). This group bias was independent of the quality of objects or their spatial location [$F_{(2, 82)} = 0.07$]. However, despite these initial group differences, both control and torpor hamsters explored the two novel objects similarly at study and test (data not shown). Moreover, the mean number and duration of target investigations decreased from study to test [Figs. 3d and e; $P < 0.001$], indicating familiarity detection and were similar in control and torpor hamsters [number, $F_{(1, 41)} = 0.72$ and duration, $F_{(1, 41)} = 0.03$].

In contrast to familiarity detection, old-new object discrimination within the test phase differed between controls and torpor hamsters. In both groups the exploration ratio changed from study to test [phase: $P < 0.001$; group: $F_{(1, 41)} = 2.05$; group x phase interaction: $F_{(1, 41)} = 1.89$]. The mean exploration ratios close to chance level (=0.33) at study, increased significantly at test (Fig. 3f; controls: $t_{(31)} = 7.52$, $P < 0.001$; torpor group: $t_{(10)} = 2.66$, $P < 0.05$, paired *t*-test). However, the increase was significantly smaller in torpor hamsters compared to controls [$t_{(19.96)} = 2.02$, $P = 0.05$; confirmed by a Kruskal-Wallis test, $H_{(1)} = 4.13$, $P < 0.05$]. In contrast to controls [$t_{(31)} = 5.69$, $P < 0.001$], in torpor hamsters the ratio difference between study and test failed to reach significance (Fig. 3f) [$t_{(10)} = 1.99$].

Locomotion of the two groups was similar at study (control, 51.3 ± 3.5, $n = 14$; torpor, 42.7 ± 2.3, $n = 7$) and at test (46.6 ± 3.2 and 50.6 ± 3.3, respectively) [group, $F_{(1, 19)} = 0.35$; phase, $F_{(1, 19)} = 0.20$; group x phase, $F_{(1, 19)} = 3.21$].

In summary, the hamsters exposed to two complex scenes sharing in common a specific object, manifested a selective reduction of exploration towards this object at test, irrespective of the experience of torpor. However, torpor hamsters showed diminished old-new object discrimination performance at retrieval.

3.2.3. Duration of daily torpor and body temperature

The duration of torpor between the study and test phase was 6.4 ± 0.4 hrs, and body temperature dropped to 20.1 ± 0.6 °C (range 18.3 - 24.5 °C). Neither minimal torpor body temperature nor torpor duration correlated with the exploration ratio in the two learning tasks (temperature: $R = 0.16$ and $R = 0.25$, duration: $R = 0.21$ and $R = -0.16$, respectively, $n = 11$ per group; Pearson product-moment correlation; pooled data of the two torpor groups: temperature: $R = 0.32$, duration: $R = - 0.17$, $n = 22$). There was a positive correlation between the duration of the interval between the study phase and the onset of the torpor episode and the ratio of exploration at test ($R = 0.46$, $F_{(20)} = 5.25$, $P = 0.032$, $n = 22$). Hence, better performance correlated with a longer consolidation interval, but not with torpor duration or body temperature.

4. Discussion

Exploratory activity towards objects is often used in rodents to investigate the memory processes involved in recognition (Steckler et al., 1998, Eacott and Norman, 2004). Recognition can be inferred from the changes in exploratory behavior towards an object encountered previously, when it is presented simultaneously with novel ones (Brown and Aggleton, 2001, Mumby, 2001).

A post-learning sleep deprivation affects familiarity and recency processes underlying object recognition in rodents differentially. The sleep deficit incurred immediately after object encoding did not interfere with the formation of a familiarity-based memory for this object. Sleep-deprived and control animals displayed similar reduced exploration of the target object at second occurrence in a simple two-object choice situation 24 hrs after encoding (Figs. 1a,b). Detection and processing of object novelty were not altered by prior sleep deprivation because the animals exhibited relatively high curiosity for the novel object at test. On the other hand, the natural attraction of hamsters for novelty may have confounded the decreased responsiveness to the target object, rendering the familiarity processing questionable. However, the significant phase-dependent decrease in novel object exploration seen in both control and sleep-deprived groups suggests that Djungarian hamsters are capable to learn in one trial an absolute class concept ‘biologically meaningless objects’ and to form a familiarity-based memory for other items belonging to the same class (Thomas, 1996). In contrast, the sleep deprivation experience led to a recency memory detriment, as seen by the diminished old-new object discrimination ability (Fig. 1c). Because the test was performed in a novel context, the use of contextual or self-motion cues to retrieve specific features of the target object was limited (Etienne and Jeffery, 2004). Therefore, the target object was the most recent to be-recognized item.

The negative effects of sleep deprivation on object recognition increased with the level of difficulty of the task. When subjected to a complex scene task in a familiar context, sleep-deprived hamsters displayed a deficit in familiarity-based memory at retrieval. They explored the target object as much as the two other novel objects (Fig. 1). In contrast, controls were able to fully recognize the familiar target (Fig. 1d,e), indicating their ability to process and identify three different objects of a spatial scene as separate entities. Therefore, sleep deprivation could have interfered with the formation of a stable memory for the whole scene, which requires the knowledge of the specific features of the objects and their spatio-temporal relationships (Graham and Gaffan, 2005). Thus, despite the presence of the target, at test sleep-deprived animals might have encoded the new scene as a novel one, due to an impaired ability to retrieve the whole scene. To test this possibility, the ability of hamsters to detect a spatial rearrangement of familiar objects in a familiar context was evaluated. Sleep deprived animals failed to discriminate between two familiar identical objects on the basis of the familiarity or novelty of their spatial location (Fig. 2e), but processed them as already seen, indicating intact familiarity encoding (Fig. 2a,b).

Thus, depending on the difficulty of the discrimination task, a sleep deprivation hampers recency memory for discrete objects and retrieval of

scene memory, while sparing object familiarity-based recognition and novelty processing.

Processes underlying new memories initially persist in a fragile state and consolidation occurs over time (McGaugh, 2000). The time window for consolidation of specific learning tasks in rats after REM sleep deprivation of different duration and timing was lasted up to 20 h (for review (Smith, 1995)). It is unknown how long the consolidation phase needs to be for the object recognition paradigm we applied. Therefore, it cannot be excluded that familiarity and novelty detection, which were intact after the early, 4-h sleep deprivation, could be sensitive to a longer sleep deprivation or a sleep deprivation performed during a later phase of memory consolidation.

The selective cognitive deficit could be either attributed to an overall lack of sleep or of slow waves during sleep, postulated to be associated with optimal conditions for synaptic downscaling (Tononi and Cirelli, 2003). Alternatively, it could result from a proactive interference effect due to the ongoing sensory stimulation during retention (e.g., (Schroeder et al., 2002, Gilbert and Kesner, 2003)). In humans, waking activities during retention can interfere with the process of consolidation and lead to a memory detriment (e.g., (Walker et al., 2003)), whereas restful waking facilitates learning and provides similar benefits as sleep (Gottselig et al., 2004).

An acute stress experience at learning can disturb memory formation and processing at retrieval (Kim et al., 2005). In mice stressful experience after encoding impaired object recognition (El Hage et al., 2004). On the other hand, in rats 48 hr sleep deprivation activated the hypothalamo-pituitary adrenal (HPA) axis, elevating plasma ACTH and corticosterone levels (Palchykova et al., 2005). However, the 4 hrs of sleep deprivation following an exposure of the hamsters to novelty did not influence the plasma levels of cortisol, corticosterone and ACTH. Therefore, it is unlikely that stress contributed to the object memory deficits seen in the hamsters after sleep deprivation.

Interestingly, the occurrence of daily torpor during retention is associated with a selective impairment in scene memory retrieval due to the deficit in target recognition within a complex spatial scene (Fig. 3). However, intact familiarity- and recency-based recognition and object novelty processing argue against potential detrimental effects of sleepiness (Deboer et al., 2000, Palchykova et al., 2003), reduced attention or vigilance associated with torpor on object information processing. The changes in body temperature during torpor did not play a major role in the memory impairment. Thus, the exploration ratios at test were independent of both body temperature reached during torpor and torpor duration. Exposure to a novelty induces long-term potentiation (LTP), a basic physiological mechanism implicated in memory formation (Li et al., 2003, Davis et al., 2004). Once established, LTP becomes insensitive to variations in temperature (Krelstein et al., 1990, Gabriel et al., 1998). It is possible that, torpor has important consequences on the brain, comparable to the transient depression of transcriptional initiation in liver during torpor (Berriel Diaz et al., 2004).

The scene memory retrieval deficit seen in torpor hamsters is analogous to that induced by sleep deprivation, suggesting the existence of detrimental factors common to both conditions. The occurrence of daily torpor later in the retention period certainly contributed to the less pronounced

cognitive impairment after torpor compared to sleep deprivation. Due to the limited amount of hamsters and the influence of the adaptation procedures on the occurrence of torpor, the timing and duration of sleep deprivation could not be matched to the torpor episode. Moreover, spontaneous torpor epochs can last up to 17 hrs that is above the duration of sleep deprivation that Djungarian hamsters tolerate without stress. These difficulties preclude the comparison between the effects of sleep deprivation and torpor.

Sleep enhances explicit recollection of words in humans, but has no effect on implicit judgement of familiarity (Drosopoulos et al., 2005). Our data are consistent with this study, and provide additional evidence that a transient sleep deficit during an early (first 4 hrs) or late time window (8 to 17 hrs) of the retention period has no effect on item familiarity and novelty processing but impairs recency and spatial scene memory retrieval of objects, depending on the difficulty of the task. Thus, the sleep deficit would not interfere with the formation of object memories but rather with cognitive abilities, which are essential for their retrieval.

Acknowledgements

We thank Dr. G. Heldmaier for providing us with the hamsters and Dr. C. Kopp for advice and comments on an early version of the manuscript. The study was supported by the Swiss National Science Foundation grants nr. 3100-053005.97/2 and 3100A0-100567/1.

References

[1] Peigneux, P.; Laureys, S.; Delbeuck, X.; Maquet, P. Sleeping brain, learning brain. The role of sleep for memory systems. Neuroreport. 2001; 12 (18):A111-124.

[2] Walker, M. P.; Stickgold, R. Sleep-dependent learning and memory consolidation. Neuron. 2004; 44 (1):121-133.

[3] Huber, R.; Ghilardi, M. F.; Massimini, M.; Tononi, G. Local sleep and learning. Nature. 2004; 430 (6995):78-81. Epub 2004 Jun 2006.

[4] Karni, A.; Tanne, D.; Rubenstein, B. S.; Askenasy, J. J.; Sagi, D. Dependence on REM sleep of overnight improvement of a perceptual skill. Science. 1994; 265 (5172):679-682.

[5] Smith, C. Sleep states and memory processes. Behav. Brain Res. 1995; 69 (1-2):137-145.

[6] Stickgold, R. Sleep: off-line memory reprocessing. Trends Cogn. Sci. 1998; 2 (12):484-492.

[7] Stickgold, R.; James, L.; Hobson, J. A. Visual discrimination learning requires sleep after training. Nat. Neurosci. 2000; 3 (12):1237-1238.

[8] Gais, S.; Plihal, W.; Wagner, U.; Born, J. Early sleep triggers memory for early visual discrimination skills. Nat. Neurosci. 2000; 3 (12):1335-1339.

[9] Harrison, Y.; Horne, J. A. The impact of sleep deprivation on decision making: a review. J. Exp. Psychol. Appl. 2000; 6 (3):236-249.

[10] Mu, Q.; Nahas, Z.; Johnson, K. A.; Yamanaka, K.; Mishory, A.; Koola, J.; Hill, S.; Horner, M. D.; Bohning, D. E.; George, M. S. Decreased cortical response to verbal working memory following sleep deprivation. Sleep. 2005; 28 (1):55-67.

[11] Ambrosini, M. V.; Giuditta, A. Learning and sleep: the sequential hypothesis. Sleep Med Rev. 2001; 5 (6):477-490.

[12] Datta, S. Avoidance task training potentiates phasic pontine-wave density in the rat: a mechanism for sleep-dependent plasticity. J. Neurosci. 2000; 20:8607-8613.

[13] Sanford, L. D.; Yang, L.; Tang, X. Influence of contextual fear on sleep in mice: a strain comparison. Sleep. 2003; 26 (5):527-540.

[14] Mavanji, V.; Siwek, D. F.; Patterson, E. H.; Spoley, E. E.; Datta, S. Effects of passive-avoidance training on sleep-wake state-specific activity in the basolateral and central nuclei of the amygdala. Behav. Neurosci. 2003; 117 (4):751-759.
[15] Hennevin, E.; Maho, C.; Hars, B.; Dutrieux, G. Learning-induced plasticity in the medial geniculate nucleus is expressed during paradoxical sleep. Behav Neurosci. 1993; 107 (6):1018-1030.
[16] Smith, C.; Rose, G. M. Evidence for a paradoxical sleep window for place learning in the Morris water maze. Physiol. Behav. 1996; 59 (1):93-97.
[17] Youngblood, B. D.; Zhou, J.; Smagin, G. N.; Ryan, D. H.; Harris, R. B. Sleep deprivation by the "flower pot" technique and spatial reference memory. Physiol. Behav. 1997; 61 (2):249-256.
[18] Smith, C. T.; Conway, J. M.; Rose, G. M. Brief paradoxical sleep deprivation impairs reference, but not working, memory in the radial arm maze task. Neurobiol. Learn. Mem. 1998; 69 (2):211-217.
[19] Graves, L. A.; Heller, E. A.; Pack, A. I.; Abel, T. Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. Learn. Mem. 2003; 10 (3):168-176.
[20] McDermott, C. M.; LaHoste, G. J.; Chen, C.; Musto, A.; Bazan, N. G.; Magee, J. C. Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons. J. Neurosci. 2003; 23 (29):9687-9695.
[21] Vertes, R. P. Memory consolidation in sleep; dream or reality. Neuron. 2004; 44 (1):135-148.
[22] Deboer, T.; Franken, P.; Tobler, I. Sleep and cortical temperature in the Djungarian hamster under baseline conditions and after sleep deprivation. J. Comp. Physiol. [A]. 1994; 174 (2):145-155.
[23] Deboer, T.; Tobler, I. Slow waves in the sleep electroencephalogram after daily torpor are homeostatically regulated. Neuroreport. 2000; 11 (4):881-885.
[24] Deboer, T.; Tobler, I. Sleep EEG after daily torpor in the Djungarian hamster: similarity to the effects of sleep deprivation. Neurosci. Lett. 1994; 166 (1):35-38.
[25] Deboer, T.; Tobler, I. Sleep regulation in the Djungarian hamster: comparison of the dynamics leading to the slow-wave activity increase after sleep deprivation and daily torpor. Sleep. 2003; 26 (5):567-572.
[26] Palchykova, S.; Deboer, T.; Tobler, I. Selective sleep deprivation after daily torpor in the Djungarian hamster. J. Sleep Res. 2002; 11 (4):313-319.
[27] Achermann, P.; Borbély, A. A. Mathematical models of sleep regulation. Front. Biosci. 2003; 8:s683-693.
[28] Tobler, I. Phylogeny of Sleep Regulation. In: Kryger, M. H.; Roth, T.; Dement, W. C., eds. Principles and practice of sleep medicine. 4th ed. Philadelphia, PA: Elsevier Saunders; 2005: p72-81.
[29] Heldmaier, G.; Ruf, T. Body temperature and metabolic rate during natural hypothermia in endotherms. J. Comp. Physiol. [B]. 1992; 162:696-706.
[30] Daan, S.; Barnes, B. M.; Strijkstra, A. M. Warming up for sleep? - ground squirrels sleep during arousal from hibernation. Neurosci. Lett. 1991; 128:265-268.
[31] Trachsel, L.; Edgar, D. M.; Heller, H. C. Are ground squirrels sleep deprived during hibernation? Am. J. Physiol. 1991; 260:R1123-R1129.
[32] Millesi, E.; Prossinger, H.; Dittami, J. P.; Fieder, M. Hibernation effects on memory in European ground squirrels (Spermophilus citellus). J. Biol. Rhythms. 2001; 16 (3):264-271.
[33] Dudai, Y. The neurobiology of consolidations, or, how stable is the engram? Annu. Rev. Psychol. 2004; 55:51-86.
[34] Brown, M. W.; Aggleton, J. P. Recognition memory: what are the roles of the perirhinal cortex and hippocampus? Nat. Rev. Neurosci. 2001; 2 (1):51-61.
[35] Ennaceur, A.; Delacour, J. A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. Behav. Brain Res. 1988; 31 (1):47-59.
[36] Steckler, T.; Drinkenburg, W.; Sahgal, A.; Aggleton, J. P. Recognition memory in rats - I. Concepts and classification. Progress Neurobiol. 1998; 54 (3):289-311.
[37] Goldman, B. D. The Siberian hamster as a model for study of the mammalian photoperiodic mechanism. Adv Exp Med Biol. 1999; 460:155-164.
[38] Figala, J.; Hoffmann, K.; Goldau, G. Zur Jahresperiodik beim Dsungarischen Zwerghamster Phodopus sungorus Pallas. Oecologia. 1973; 12:89-118.

[39] Tobler, I.; Deboer, T.; Fischer, M. Sleep and sleep regulation in normal and prion protein-deficient mice. J. Neurosci. 1997; 17 (5):1869-1879.
[40] Tobler, I.; Gaus, S. E.; Deboer, T.; Achermann, P.; Fischer, M.; Rulicke, T.; Moser, M.; Oesch, B.; McBride, P. A.; Manson, J. C. Altered circadian activity rhythms and sleep in mice devoid of prion protein. Nature. 1996; 380 (6575):639-642.
[41] Huber, R.; Deboer, T.; Tobler, I. Topography of EEG dynamics after sleep deprivation in mice. J. Neurophysiol. 2000; 84:1888-1893.
[42] Ruf, T.; Steinlechner, S.; Heldmaier, G. Rhythmicity of body temperature and torpor in the Djungarian hamster, *Phodopus sungorus*. In: Malan, A.; Canguilhem, B., eds. Living in the cold. 2nd ed. John Libbey Eurotext Ltd; 1989: p53-61.
[43] Eacott, M. J.; Norman, G. Integrated memory for object, place, and context in rats: a possible model of episodic-like memory? J. Neurosci. 2004; 24 (8):1948-1953.
[44] Mumby, D. G. Perspectives on object-recognition memory following hippocampal damage: lessons from studies in rats. Behav. Brain Res. 2001; 127 (1-2):159-181.
[45] Thomas, R. K. Investigating cognitive abilities in animals: unrealized potential. Brain Res Cogn Brain Res. 1996; 3 (3-4):157-166.
[46] Etienne, A. S.; Jeffery, K. J. Path integration in mammals. Hippocampus. 2004; 14 (2):180-192.
[47] Gaffan, E. A.; Healey, A. N.; Eacott, M. J. Objects and positions in visual scenes: effects of perirhinal and postrhinal cortex lesions in the rat. Behav. Neurosci. 2004; 118 (5):992-1010.
[48] McGaugh, J. L. Memory--a century of consolidation. Science. 2000; 287 (5451):248-251.
[49] Tononi, G.; Cirelli, C. Sleep and synaptic homeostasis: a hypothesis. Brain Res. Bull. 2003; 62 (2):143-150.
[50] Gilbert, P. E.; Kesner, R. P. Recognition memory for complex visual discriminations is influenced by stimulus interference in rodents with perirhinal cortex damage. Learn Mem. 2003; 10 (6):525-530.
[51] Schroeder, J. P.; Wingard, J. C.; Packard, M. G. Post-training reversible inactivation of hippocampus reveals interference between memory systems. Hippocampus. 2002; 12 (2):280-284.
[52] Walker, M. P.; Brakefield, T.; Hobson, J. A.; Stickgold, R. Dissociable stages of human memory consolidation and reconsolidation. Nature. 2003; 425 (6958):616-620.
[53] Gottselig, J. M.; Hofer-Tinguely, G.; Borbély, A. A.; Regel, S. J.; Landolt, H. P.; Retey, J. V.; Achermann, P. Sleep and rest facilitate auditory learning. Neuroscience. 2004; 127 (3):557-561.
[54] Kim, J. J.; Diamond, D. M. The stressed hippocampus, synaptic plasticity and lost memories. Nat. Rev. Neurosci. 2002; 3 (6):453-462.
[55] El Hage, W.; Peronny, S.; Griebel, G.; Belzung, C. Impaired memory following predatory stress in mice is improved by fluoxetine. Prog Neuropsychopharmacol Biol Psychiatry. 2004; 28 (1):123-128.
[56] Meerlo, P.; Koehl, M.; van der Borght, K.; Turek, F. W. Sleep restriction alters the hypothalamic-pituitary-adrenal response to stress. J. Neuroendocrinol. 2002; 14 (5):397-402.
[57] Deboer, T.; Vyazovskiy, V. V.; Tobler, I. Long photoperiod restores the 24-h rhythm of sleep and EEG slow-wave activity in the Djungarian hamster (*Phodopus sungorus*). J. Biol. Rhythms. 2000; 15 (5):429-436.
[58] Palchykova, S.; Deboer, T.; Tobler, I. Seasonal aspects of sleep in the Djungarian hamster. BMC Neurosci. 2003; 4 (1):1-9.
[59] Davis, C. D.; Jones, F. L.; Derrick, B. E. Novel environments enhance the induction and maintenance of long-term potentiation in the dentate gyrus. J Neurosci. 2004; 24 (29):6497-6506.
[60] Li, S.; Cullen, W. K.; Anwyl, R.; Rowan, M. J. Dopamine-dependent facilitation of LTP induction in hippocampal CA1 by exposure to spatial novelty. Nat Neurosci. 2003; 6 (5):526-531.
[61] Krelstein, M. S.; Thomas, M. P.; Horowitz, J. M. Thermal effects on long-term potentiation in the hamster hippocampus. Brain Res. 1990; 520 (1-2):115-122.
[62] Gabriel, A.; Klussmann, F. W.; Igelmund, P. Rapid temperature changes induce adenosine-mediated depression of synaptic transmission in hippocampal slices from

rats (non-hibernators) but not in slices from golden hamsters (hibernators). Neuroscience. 1998; 86 (1):67-77.
[63] Berriel Diaz, M.; Lange, M.; Heldmaier, G.; Klingenspor, M. Depression of transcription and translation during daily torpor in the Djungarian hamster (Phodopus sungorus). J Comp Physiol [B]. 2004; 174 (6):495-502.
[64] Drosopoulos, S.; Wagner, U.; Born, J. Sleep enhances explicit recollection in recognition memory. Learn. Mem. 2005; 12 (1):44-51.

Paper 4

Sleep deprivation impairs object recognition in mice

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Published in Neurobiology of Learning and Memory 2006: 85(3), 263-271

Abstract

Many studies in animals and humans suggest that sleep facilitates learning, memory consolidation and retrieval. Moreover, sleep deprivation (SD) incurred after learning, impaired memory in humans, mice, rats and hamsters. We investigated the importance of sleep and its timing in an object recognition task in OF1 mice subjected to 6 h SD either immediately after the acquisition phase (0-6 SD) or 6 h later (7-12 SD), and in corresponding undisturbed controls. Motor activity was continuously recorded with infra-red sensors.

All groups explored two familiar, previously encountered objects to a similar extent, both at the end of the acquisition phase and 24 h later during the test phase, indicating intact familiarity detection. During the test phase 0-6 SD mice failed to discriminate between the single novel and the two familiar objects. In contrast, the 7-12 SD group and the two control groups explored the novel object significantly longer than the two familiar objects. Plasma corticosterone levels determined after SD did not differ from time-matched undisturbed controls, but were significantly below the level measured after learning alone. ACTH did not differ between the groups. Therefore, it is unlikely that stress contributed to the memory impairment.

We conclude that the loss of sleep and the activities the mice engaged in during the SD, impaired recognition memory retrieval, when they occurred immediately after acquisition. The delayed SD enabled memory consolidation during the 6 h when the mice were allowed to sleep, and had no detrimental effect on memory. Neither SD schedule impaired object familiarity processing, suggesting that only specific cognitive abilities were sensitive to the intervention. Sleep may either actively promote memory formation, or alternatively, sleep may provide optimal conditions of non-interference for consolidation.

Key words: Sleep deprivation; Object recognition; Familiarity; Stress; Rest; Motor activity

1. Introduction

There is increasing evidence that sleep may be important for learning and memory, whereas a sleep deficit results in performance impairment both in rodents and humans (for review (Stickgold, 1998, Walker, 2004). However, the role of sleep in memory formation is complex and appears to depend on the nature of the task (e.g., (Graves et al., 2003). Moreover, there are data indicating that sleep should occur within a specific time window following upon the training or acquisition phase in order to facilitate learning effectively (e.g., (Pearlman, 1973, Smith and Butler, 1982, Smith et al., 1991). Thus, 4 h of REM sleep deprivation immediately following training resulted in learning impairment in the hidden platform of the Morris water maze, but not in the visual platform (Smith and Rose, 1996). The same duration of REM sleep deprivation performed after acquisition in the eight-arm radial maze task resulted in a deficit of spatial reference memory, whereas working memory was intact (Smith et al., 1998). Recent data show that Fisher rats deprived from REM sleep for 4 h after training, switch from a spatial to a non-spatial strategy to solve a complex associative learning task (Bjorness et al., 2005). Moreover, 5 h total sleep deprivation in C57BL/6 mice impaired contextual but not cued fear conditioning, when the SD was scheduled immediately after the acquisition phase (Graves et al., 2003). In contrast, delayed REM sleep deprivation (hours 4-8, 8-12 or 13-24 after acquisition) or delayed total sleep deprivation (hours 5-10) had no effect on memory (Smith and Rose, 1996, Smith et al., 1998, Graves et al., 2003, Bjorness et al., 2005). Taken together, these data indicate that sleep at the appropriate time, may facilitate and optimize memory formation of certain tasks. On the other hand, the fundamental relevance of sleep in memory processing has been questioned (reviewed in (Vertes, 2004, Coenen, 2005, Doyon et al., 2005, Korman et al., 2005, Schredl, 2005, Siegel, 2005, Vertes, 2005). Important arguments are the specificity of the tasks that seem to profit from sleep, contrasting a global role of sleep in memory consolidation, and the limitations of comparing results obtained in humans and animals.

The one-trial object recognition task, originally developed for rats (Ennaceur and Delacour, 1988) and applied in several mouse studies (e.g., (Dodart et al., 1997, Genoux et al., 2002), provides a useful behavioral paradigm to investigate the effects of sleep loss on memory consolidation and retrieval. Animals have to learn to recognize biologically meaningless objects or their spatial location, and should be able to retrieve the object or location information in a complex spatial scene when tested 24 h later. It was suggested that memories of objects in rodents can be compared to human episodic-like memory (Dere et al., 2004, Blackstone et al., 2005).

To our knowledge the role of sleep on recognition memory has not been investigated in animals. Therefore, it is unknown whether there is a specific timing of sleep that may be more important for consolidation or retention of recognition memories. To test whether undisturbed sleep occurring immediately after learning is critical and sufficient for consolidation of object recognition, we subjected mice to 6 h total SD by “gentle handling” either immediately (0-6 SD) or 6 h after the end of an acquisition phase (7-12 SD).

Sleep deprivation has been considered to be stressful for animals (Horne and McGrath, 1984). Since stress may be a cause for memory impairment, we measured the plasma levels of the stress hormones corticosterone and ACTH in mice subjected to acquisition alone and in mice subjected to acquisition followed by SD. To evaluate the effectiveness of SD and the effects of learning and SD on rest, motor activity was recorded with infra-red (IR) sensors for several days before the learning task and throughout the experimental days.

2. Materials and Methods

2.1. Animals

The Cantonal Veterinary Office of Zürich approved all experimental procedures. The experiments were performed in OF1 mice because a detailed analysis of their sleep and the effects of 5 h sleep deprivation were reported recently (Kopp et al., 2002) and OF1 mice have been used in similar object recognition tests (Bour et al., 2004). Adult male outbred OF1 mice (*n* = 89), weighing 41 ± 5 g (SD), were kept individually in Macrolon cages (36 x 20 x 35 cm) with food and water available *ad libitum.* The animals were maintained on a 12 h light : 12 h dark cycle (light onset at 9 A.M.; ~ 30 lux) at 23°C ambient temperature. All behavioral tests were performed at the age of 15 - 16 wks under dim red lighting conditions (< 5 lux), starting 30 - 105 min before light onset.

2.2. Activity recordings

Passive IR activity was recorded continuously throughout the experiment. The sensor mounted above the cage generated a signal (activity counts) in response to spatial movements of the mouse. Counts were integrated over consecutive 1-min epochs and stored on a computer, as described previously (Tobler et al., 1996b); Chronobiology Kit, Stanford Software system, Stanford, CA). The effects of SD or learning on motor activity, rest and the number and duration of rest epochs were investigated based on 1-h or 6-h intervals and compared to baseline 10-day mean values. Two aspects of rest-activity were evaluated: 1. intensity of activity during activity bouts (total activity counts within an hour divided by the number of 1-min epochs with activity above zero); 2. duration of rest (defined as the number of 1-min epochs with activity counts equal zero).

2.3. Sleep deprivation procedure

Mice were subjected to 6 h SD starting either immediately after the acquisition phase (0-6 SD, n=9), coinciding with onset of the 12-h light period, or 6 h later (7-12 SD, n=9). Animals of the two corresponding time of day control groups (n=8-9 per group) were returned to their home cage immediately after acquisition and left undisturbed. The order of testing of SD and control animals was randomized. SD was achieved by "gentle handling" as described previously (Tobler et al., 1997a). During the SD procedure the mice were

provided with familiar nesting material (tissues). No novel objects were introduced.

2.4. Behavioral tasks

The object and location recognition task comprised an acquisition phase, delay phase, and test phase. All animals were individually familiarized with the experimental context but without objects for 15 min at the end of the 12-h dark period on two consecutive days, immediately preceding the acquisition phase. The floor of a square enclosure (grey plastic, 75 x 75 x 37 cm) was covered with soiled wood chippings collected from cages of several mice, ensuring saturation with odors of conspecifics. No specific spatial cues were provided within the enclosure. Nine sets of 3-4 identical objects (volume approximately 16 to 18 cm^3) differing in shape, color and texture and with no biological relevance were kept in the boxes with soiled wood chippings collected from cages of several mice. Objects and their spatial location were randomized among the animals. Video recordings were obtained throughout the acquisition and test phase.

To test memory for a complex scene, four groups of mice (SD 0-6, SD 7-12 and corresponding controls) were given the opportunity to explore a triplet of novel objects for five times 5 min with 15-min intervals between exposures during the acquisition phase at the end of the dark period (Fig. 1). Acquisition was followed by a 24-h delay phase during which the mice either experienced a 6-h sleep deficit followed by undisturbed sleep during the remaining 12 - 18 h, or were immediately returned to their home cage and left undisturbed (controls). A 24-h retention period was chosen to avoid a circadian effect on performance (e.g. (Chaudhury and Colwell, 2002) and to provide sufficient time for recovery (Kopp et al., 2002). In the subsequent test phase, the mice were exposed for 5 min to a new triplet of objects: two objects encountered previously in the acquisition phase were replaced by an identical copy (targets), and presented together with a novel object. If recognition memory was intact, the mice were expected to spend more time exploring the novel object (Ennaceur and Delacour, 1988).

To test memory for the spatial location of objects, a 5th group of mice (n = 10) was allowed to investigate two identical copies of a novel object for five times 5 min with 15-min intervals between exposures during acquisition (Fig. 1 B). The objects were placed in two adjacent corners of the square enclosure. In the 5-min test phase 24 h later, the objects were replaced by two identical copies, one of which was placed in its original location (target), the other in a different corner. The position of the target was counter-balanced among individuals. If the animals perceived the spatial rearrangement of the two familiar objects, they were expected to spend more time exploring the object in the novel location (Ennaceur and Meliani, 1992).

2.5. Plasma ACTH and corticosterone assays

An additional batch of mice (n = 24; 14 - 15 wks old) kept in the same environment and conditions as the experimental groups, was used to collect

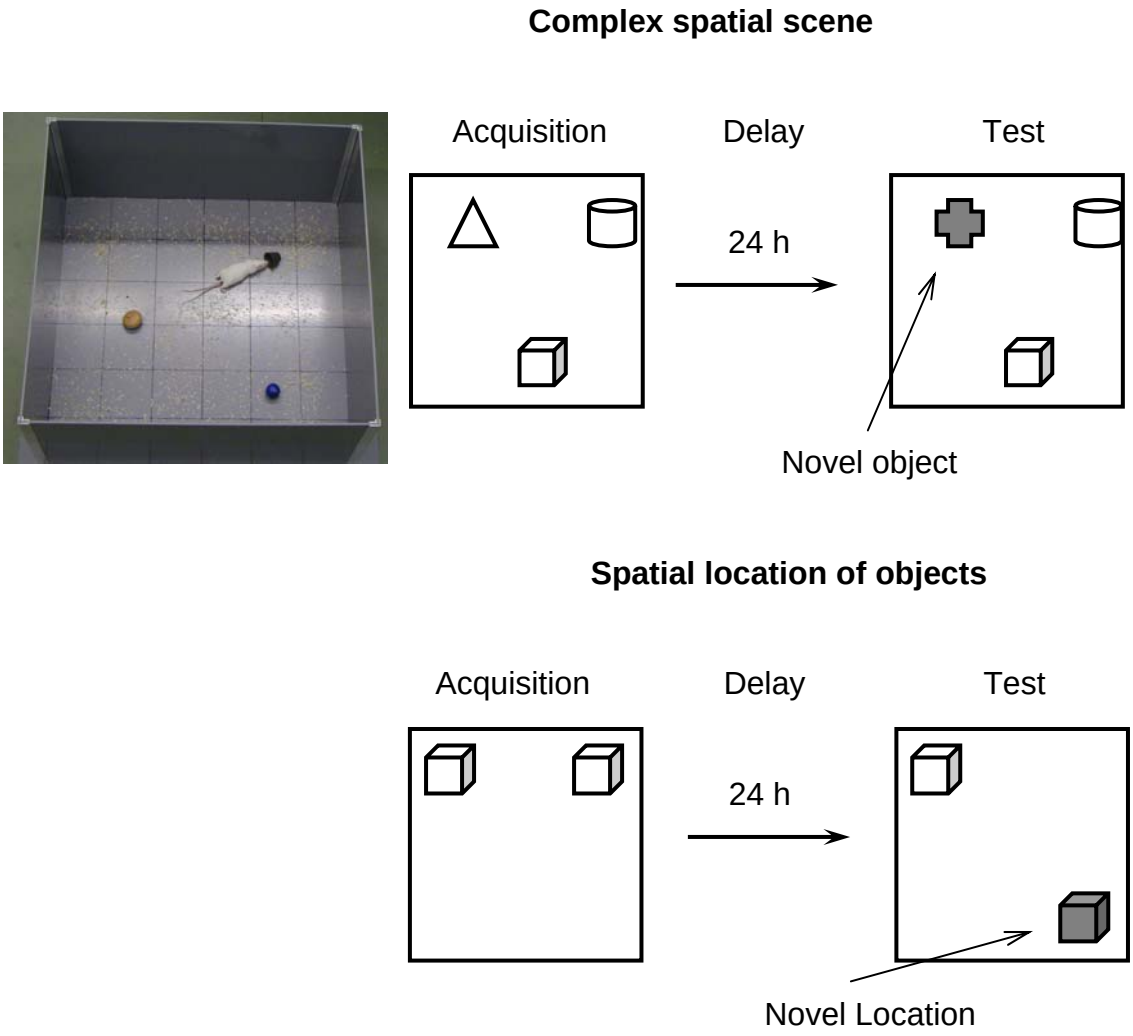

Figure 1. Photo and scheme of the complex spatial scene version of the object recognition task. During the acquisition phase mice were exposed to a triplet of novel objects for five times 5 min with 15-min intervals between exposures (top) or two identical copies of a novel object placed in two adjacent corners of the enclosure (bottom). Acquisition was followed by a 24-h delay phase. During the test phase, the mice were exposed for 5 min to a new triplet of objects: two objects encountered previously during acquisition were replaced by an identical copy and presented together with a novel object (top) or the objects were replaced by two identical copies, one of which was placed in its original location, the other in a different corner (bottom).

blood for measurement of stress hormones after learning and SD. Animals were subjected to acquisition of the complex scene learning task, and then subdivided. One group was subjected to 6 h SD, starting as in the memory experiments, immediately after acquisition and killed at the end of SD ('Acquisition+6hSD'; *n* = 8), and two groups were killed immediately ('Acquisition'; n = 8) or 6 h after the acquisition phase ('Acquisition+6h'; *n* = 8). Undisturbed controls were kept in individual home-cages in a separate room and killed at the same time of day as the 'Acquisition' (Control 1; *n* = 10) or 'Acquisition+6hSD' group (Control 2; *n* = 11). An additional group of mice was immobilized for 30 min at the same time of day as the 'Acquisition+6hSD' group and killed thereafter ('Immobilized'; *n* = 9).

The mice were decapitated and trunk blood was collected in K2E EDTA K2 tubes (Vacuette® 2 ml, Greiner Bio-One Vacuette Schweiz GmbH) kept on

ice and then centrifuged at 2600 *g* at 4 °C for 15 min. The supernatant was stored at -80 °C in cryotubes for later analysis. Plasma corticosterone and ACTH concentrations were determined by radioimmunoassay (corticosterone kit, MP Biomedicals, Costa Mesa, USA; ACTH kit, Nichols Institute Diagnostics, Bad Vilbel, Germany).

2.6. Groups, variables and statistics

Interactions with objects were quantified by visual off-line scoring of the video tapes by a trained observer. The exploratory behavior towards objects was quantified as time spent at a distance < 2 cm and/or contact with the objects. Circling around or sitting on the objects was not considered. For the test phase, an object exploration ratio was calculated. It was defined as the time spent on exploration of the novel object, divided by the time spent exploring the novel object plus the mean time exploring the two familiar target objects. For the acquisition phase, the same ratio was calculated for the object occupying the same location as the novel object during the test phase. A ratio of 0.5 indicated similar exploration of the novel and familiar target objects, corresponding to chance; a ratio > 0.5 indicated a higher exploration of the novel object.

A two-way ANOVA with between subject factor 'group' and within subject factor 'object', 'phase (acquisition, test)' or 'session (1, 5)' was used to analyze behavioral data and one-way ANOVA (factor 'group') and Kruskal-Wallis test (when not normally distributed; normality was tested with the procedure Univariate; SAS) was used for plasma corticosterone levels (after decimal logarithm transformation), and plasma ACTH levels. Activity and rest were analyzed with two-way ANOVA for repeated measures, within subject factor 'day (baseline vs. acquisition)' and 'interval (1- or 6-h values)'. When significance was reached, post-hoc testing was performed with the Tukey test, unpaired or paired *t*-tests for between and within group comparisons, respectively, and non-parametric Kruskal-Wallis and Wilcoxon signed-rank tests for samples that were not normally distributed. For object exploration, the mean for the two target objects was used, after ensuring by statistical testing that exploration at study or test did not differ significantly.

3. Results

3.1. Object recognition in the complex scene in OF1 mice

During the acquisition phase, the two control groups explored each of the three objects to the same extent ['object' $F(2,30)=0.77$, 'group' $F(1,15)=1.63$, 'object x group' $F(2.30)=0.01$] and displayed a reduction in their exploration [Fig. 2 A; 'session: 1 vs 5' $F(1,15)=8.67$, $p<.006$, 'group' $F(1,15)=0.13$, 'group x session' $F(1,15)=0.04$].

During the test phase (Fig. 2 B), both control groups spent more time exploring the novel object compared to the two familiar objects [$p<.05$ Tukey test after two-way ANOVA factor 'object' $F(1,15)=12.33$ was $p<.001$]. In addition, there was a significant group effect ['group' $F(1,15)=6.18$, $p<.019$]. Thus, total object exploration was lower in controls of the 7-12 SD than those of the 0-6 SD ($p<.05$, Tukey test). However, the ANOVA interaction 'group x

objects' was n.s. [F(1,15)=2.39], indicating that the controls did not differ in their discrimination performance. The analysis of the exploration ratio confirmed this finding (Fig. 2 C). Both control groups showed a similar increase in the exploration ratio from acquisition to test [p<.05 Tukey, 'phase' F(1,15)=16.33, p<.0003, 'group' F(1,15)=0.50, 'group x phase' F(1,15)=0.22].

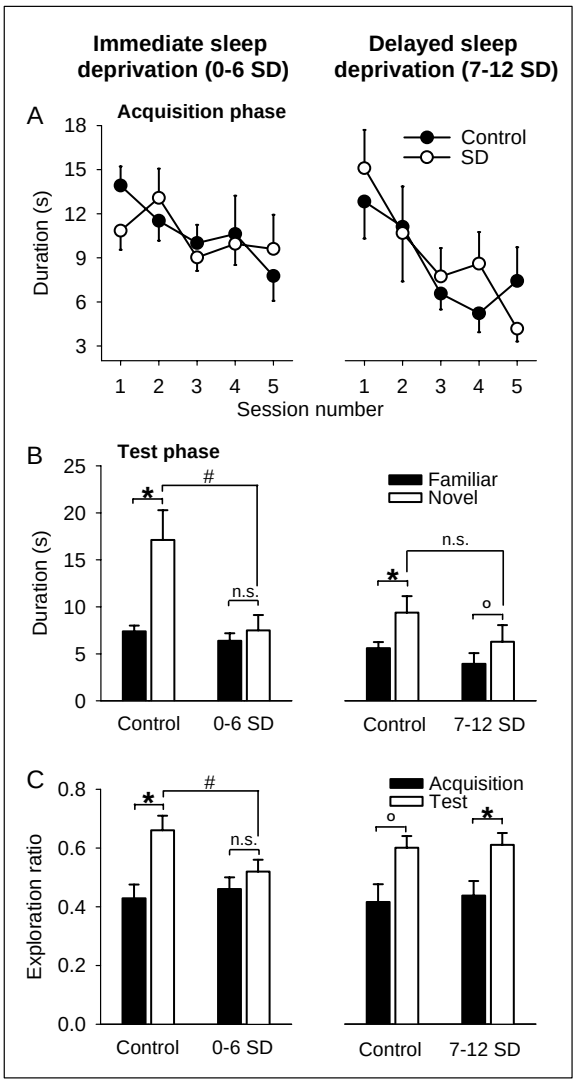

Figure 2. Effects of 6 h sleep deprivation (SD) performed either immediately after acquisition (0-6 SD; left) or 6 h later (7-12 SD; right) on object recognition. Left: (A) Time course of object exploration (mean duration in seconds per object) during the acquisition phase in the 0-6 SD and control mice ['session' F(1,16)=4.67, p<.04; 'group' F(1,16)=0.13 and 'group x session' F(1,16)=2.05; n = 9 per group]. (B) Time spent exploring the familiar objects (mean of two objects) and a single novel object during the test phase. Control mice explored the novel object longer than the familiar objects (*p<.05, paired t-test), whereas 0-6 SD mice did not. The 0-6 SD group explored the novel object less than the controls ($^{\#}p$<.05, Kruskal-Wallis). (C) The exploration ratio during the acquisition (mean per session) and test phase in the two groups (acquisition vs. test: *p<.05, paired t-test; 0-6 SD vs. controls: $^{\#}$p<.05, unpaired t-test).
Right: (A) Time course of object exploration during the acquisition phase in 7-12 SD (n = 9) and control mice (n = 8) ['session' F(1,15)=14.22, p<.0007, 'group' F(1,15)=0.05 and 'group x session' F(1.15)=1.62]. (B) Time spent exploring the familiar objects and a single novel object during the test phase (controls, t=2.57, *p<.05; 7-12 SD, t=1.97, °p=.08). (C) The exploration ratio during the acquisition and test phase in the two groups (acquisition vs. test: controls, t=1.94, °p=.09; 7-12 SD, t=2.76, *p<.05). Mean values ± SE.

3.2. A 6-h sleep deprivation immediately after the acquisition phase led to object recognition impairment in the complex scene

During the acquisition phase, each of the three objects was explored to the same extent ['object' F(2,32)=0.15], by both groups ['group: control, SD' F(1,16)=0.86, 'group x object' F(2,32)=0.74]. Moreover, the time the animals spent exploring the objects decreased progressively in both groups (Fig. 2 A left). The comparison of time spent on the two (familiar) target objects during the test phase compared to time spent on the same objects at the end of the acquisition phase did not differ within either group ['phase: last 5 min of acquisition vs. test' F(1,16)=1.02]. This result indicates that in both groups

familiarity detection was intact [‘group’ $F(1,16)=0.28$ and ‘group x phase’ $F(1,16)=1.17$].

In the test phase, a significant detrimental effect of SD was evident by the failure of the sleep deprived mice to discriminate between the familiar and novel objects. Thus, the SD group explored the novel object to the same extent as the two familiar targets (Fig. 2 B left), whereas the control group spent significantly more time exploring the novel object. Also the group comparison showed an impairment in the SD group, by their significantly lower exploration of the novel object compared to the control group, while both groups explored the familiar objects to a similar extent [Fig. 2 B left; duration of exploration: ‘group’ $F(1,16)=8.26$, $p<.007$; ‘group x object: novel, mean or two familiar’ $F(1,16)=5.43$, $p<.03$].

The exploration ratio confirmed the failure of the SD mice to discriminate between the novel and familiar objects: in the SD mice the ratio did not differ between acquisition and test (Fig. 2 C left), while it increased significantly in controls. The groups did not differ during acquisition (Fig. 2 C left, black bars), while at test (white bars) the ratio was significantly lower in the SD group than in the controls [‘phase’ $F(1,16)=9.49$, $p<.0042$, ‘group x phase’ $F(1,16)=3.47$, $p<.0718$].

In summary, SD during the first 6 h after exposure to three objects in the acquisition phase impaired the ability to discriminate between two objects encountered previously and a novel one.

3.3. No effect of a delayed sleep deprivation

As in the previous experiment, during the acquisition phase each of the three objects was explored to the same extent [‘object’ $F(2,30)=0.57$] by both groups [‘group: control, SD’ $F(1,15)=0.12$, ‘group x object’ $F(2,30)=0.19$]. Again, there was a similar progressive reduction in the time the animals spent exploring the objects (Fig. 2 A right). The time spent on the two (familiar) target objects during the test phase was similar to time on the same objects at the end of the acquisition [‘phase’ $F(1,15)=0.84$]. This result was similar between the groups [‘group’ $F(1,15)=2.49$, ‘group x phase’ $F(1.15)=0$], indicating intact familiarity detection, despite the SD.

In contrast to the 0-6 SD experiment, there was no effect of SD in the 7-12 SD paradigm during the test phase. Thus, both control and sleep-deprived mice spent significantly more time exploring the novel object compared to the two familiar objects [Fig. 2 B right; $p<.05$ Tukey test, ‘object’ $F(1,15)=4.62$, $p<.039$]. No group difference occurred [‘group’ $F(1,15)=2.76$, ‘group x object’ $F(1,15)=0.26$].

The comparison of the mean exploration ratio within the acquisition phase and test showed a significant increase from acquisition to test in both groups [Fig. 2 C right; $p<.05$, Tukey, ‘phase’ $F(1,15)=13.86$, $p=.0008$]. There was no group difference either within the acquisition or test phases [‘group’ $F(1,15)=0.15$, ‘group x phase’ $F(1,15)=0.01$].

In summary, the 6-h SD experienced 6 h after the acquisition phase did not affect the ability to discriminate between a novel and two previously encountered objects.

3.4. Memory for spatial location of object

As was expected, the mice explored the two identical novel objects to the same extent during the acquisition phase (total exploration during entire acquisition phase: 75.9 s ± 28.1 (SE) and 79.8 s ± 21.9, n.s., Wilcoxon signed-ranks test). During the test phase, in contrast to the expectation that the object in the novel location would be explored more than the one in the same location as previously, there was no difference in exploration of two duplicates of the original objects, when one of them occupied a novel location (5-min test phase: 19.5 ± 6.0 s and 20.9 ± 7.9 s, n.s., Wilcoxon). Thus, even the undisturbed control group was unable to detect the spatial rearrangement of two already seen objects under this training paradigm after the 24-h delay. Therefore, the effect of SD was not evaluated in this task.

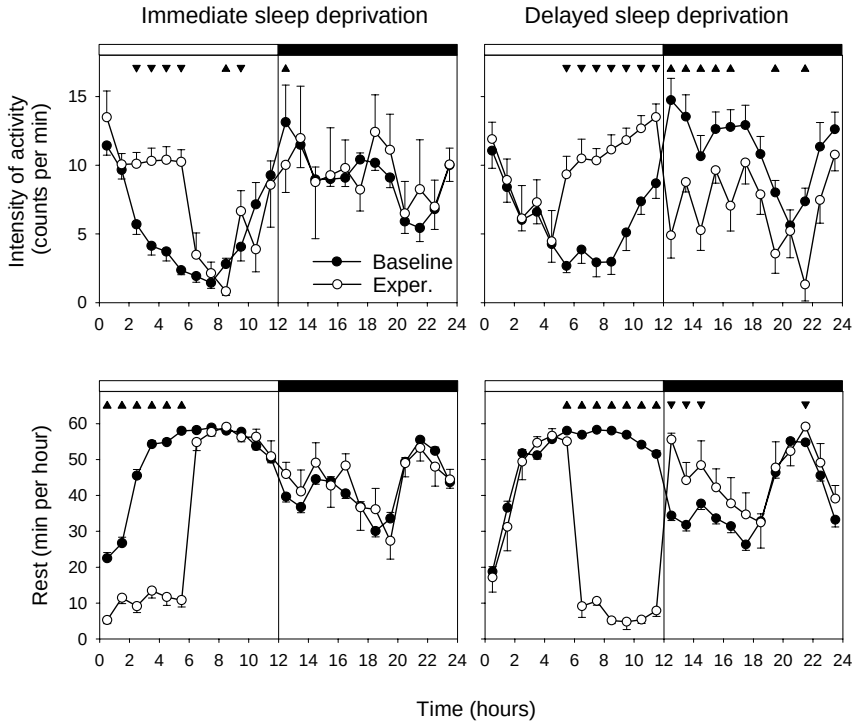

Figure 3. Top: time course of infra-red (IR) activity intensity (1-h values, mean ± SE, arbitrary units, IR-counts/number of 1-min epochs with counts > 0) during baseline (average of 10 undisturbed days) and the sleep deprivation (SD=Exper.) day (left: 0-6 SD ['day x interval' $F(23,368)=1.99$, $p<.0048$]; right: delayed 7-12 SD ['day x interval' $F(23,368)=9.30$, $p<.0001$]; $n=9$ per group). Bottom: rest (number of epochs with activity=0 per hour) (left: 0-6 SD ['day x interval' $F(23,368)=15.0$, $p<.0001$]; right: 7-12 SD ['day x interval' $F(23,368)=23.76$, $p<.0001$]). Triangles: Exper. vs. baseline, $p<.05$, paired *t*-test.

3.5. Activity and rest

The loss of rest during the 6-h SD interval was larger in the delayed SD paradigm compared to the immediate SD [$t(16)=12.39$, $p<.0001$, unpaired t-test; number of rest epochs during the SD vs. the corresponding baseline: 0-6 SD, 261.9 ± 3.4 min, 61.7 ± 6.9 min; 7-12 SD, 335.8 ± 1.5 min, 42.8 ± 7.4 min, $p<.0001$, paired t-test after 'day x 6-h interval' $F(3,48)=78.77$ and $F(3,48)=92.37$, $p<.0001$, respectively]. During recovery after 0-6 SD, changes in intensity of activity were minor and the amount of rest was unchanged (Fig. 3). In contrast, after 7-12 SD, immediate and prolonged changes occurred:

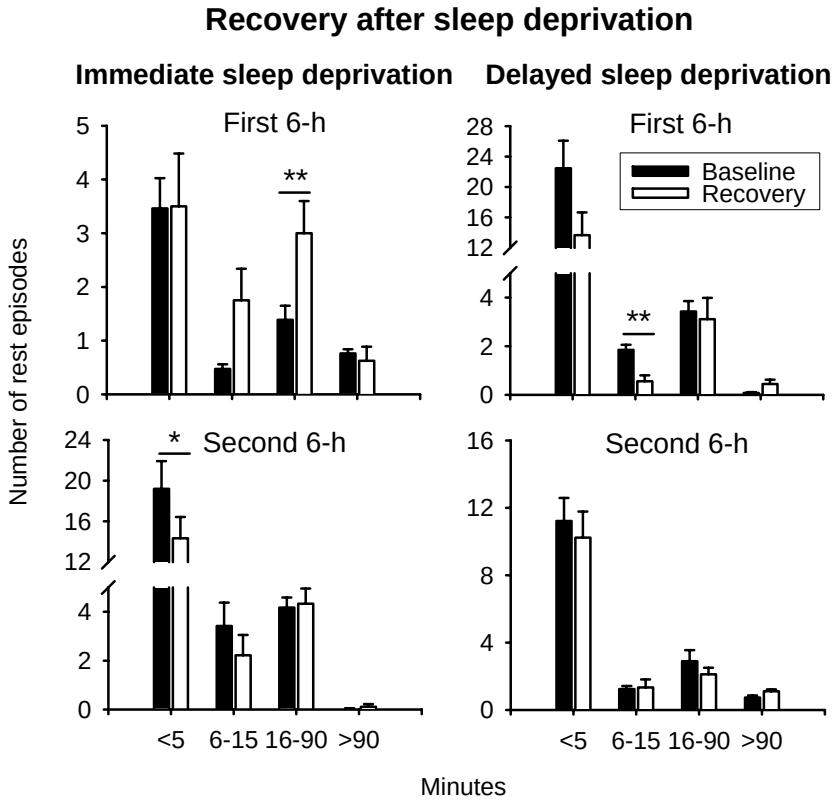

Figure 4. Number of rest episodes (duration in min: <5, 6-15, 16-90, and >90) after sleep deprivation (SD) performed immediately after acquisition (left panels) or 6 h later (delayed SD; right panels) and during corresponding baseline intervals (average of 10 undisturbed days; n = 9 per group). Mean values for 6-h intervals ± SE. Recovery vs. baseline: *p<.05, **p<.005; 0-6 SD (6-15 min, first 6 h), 7-12 SD (>90 min, first and second 6 h), p=.06, paired t-test.

activity was reduced during almost the entire subsequent dark period, while rest was significantly increased for 3 h (Fig. 3). In the control groups, neither activity nor rest differed significantly between baseline and the experimental days (0-6 SD control: activity, 'day' $F(1,16)=0.0$, 'day x 1-h interval' $F(23,32)=0.99$, rest, 'day' $F(1,16)=0.21$, 'day x 1-h interval' $F(23,32)=0.91$; 7-12 SD control: activity, 'day' $F(1,14)=0.08$, 'day x 1-h interval' $F(23,28)=0.70$, rest, 'day' $F(1,14)=0.28$, 'day x 1-h interval' $F(23,28)=0.93$).

Analysis of rest episode duration during recovery revealed that the immediate SD (SD 0-6) led to a significant increase in the number of long rest episodes in the first 6-h interval after SD (Fig. 4 left). After the delayed SD (SD 7-12), the number of short rest episodes decreased significantly (Fig. 4 right). The immediate SD still exerted a significant effect in the second 6-h recovery interval, where short rest episodes were significantly decreased.

During the last 90 min of the baseline dark period, corresponding to the acquisition phase on experimental days, rest comprised 61.7 ± 1.4 min (mean of 10 days). The acquisition phase was followed by a significant consolidation of rest. Thus, in the 6-h interval following acquisition the number of rest episodes longer than 16 min was increased from 1.60 ± 0.11 to 2.19 ± 0.22 (t=2.72, p<.05, paired t-test) at the cost of short rest episodes (<5 min, from 14.61 ± 1.36 to 11.27 ± 1.00, t=3.19, p<.005) (n=26; pooled values of the three groups undisturbed immediately after acquisition: two controls and 7-12 SD).

3.6. Stress hormones

Corticosterone levels at the end of the acquisition phase ('Acquisition') were significantly above the values of the undisturbed time-matched 'Control 1' (Fig. 6 left; $p<.0001$, unpaired t-test). SD following acquisition induced a mild but significant increase of plasma corticosterone above the levels of time-matched mice subjected to the acquisition phase only [$p<.05$, Tukey after 'group (3-6)' $F(3,32)=45.49$, $p<.0001$]. However, neither of these two groups differed from the undisturbed time-matched controls. Also, the corticosterone level at the end of SD following the acquisition phase was significantly lower than immediately after acquisition alone ($p<.05$, Tukey). For comparison, immobilization stress led to a major increase of plasma corticosterone above the amount encountered in all other groups ($p<.05$, Tukey), with the exception of the level immediately following the acquisition phase.

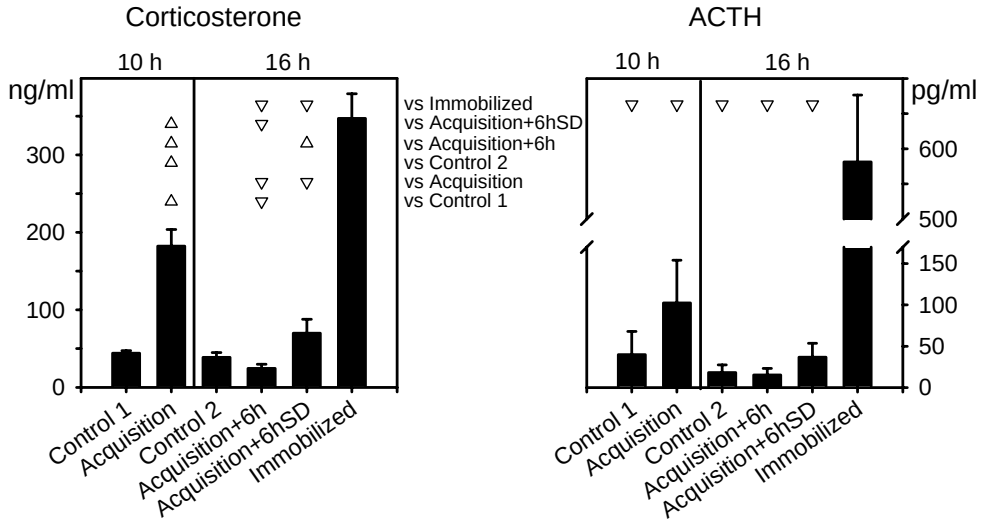

Figure 5. Plasma levels of corticosterone (ng/ml) and ACTH (pg/ml) in mice subjected to the acquisition phase of the object recognition task and 6 h sleep deprivation (SD). Mice were sacrificed at two time points: 10 and 16 h (Light from 10:00-22:00 h). Acquisition alone ('Acquisition'; $n = 8$) induced a large increase in corticosterone which recovered during the subsequent 6 h in mice left undisturbed after the acquisition phase ('Acquisition + 6 h'; $n = 8$), but also in the mice sleep deprived ('Acquisition + 6 h SD'; $n = 8$). Control 1 and 2: time-matched undisturbed groups ($n = 10$-11); 'Immobilized': mice immobilized for 30 min ($n = 9$). Mean values ± SE. Triangles above bars indicate significant differences between the bar and other groups (legend on the side): $p<0.05$, Tukey test. Orientation of triangles indicates the direction of deviation.

The levels of plasma ACTH attained after immobilization were significantly above the values of the other groups (Fig. 5 right; $p<.05$, Tukey). There were no further differences between the groups.

4. Discussion

A period of sleep deprivation following immediately upon an acquisition phase caused a significant retrieval detriment in a complex spatial scene paradigm 24 h later. This deficit in object discrimination was restricted to the group of

mice subjected to SD during the first 6 h following acquisition, since both the controls that were allowed to sleep, as well as the mice subjected to a similar but delayed SD had no such deficit (Fig. 2 C). Apparently, only the early intervention interfered with the process of memory consolidation necessary for optimal performance, while 6 h of undisturbed sleep following immediately upon the acquisition phase were sufficient for memory consolidation. Our results are consistent with the negative or modulating effects on performance of SD timed immediately after acquisition in contextual fear conditioning in C57BL/6 mice (Graves et al., 2003), in an object recognition task in hamsters (Palchykova et al., 2005) and in an associative learning task in rats (Graves et al., 2003, Bjorness et al., 2005). Taken together the results confirm that the timing of sleep is crucial for its beneficial effect on memory consolidation and retrieval.

Many studies have suggested that sleep-specific physiological processes may actively facilitate learning. Thus, correlation analysis of neuronal activity recorded in freely moving rodents shows that distinct firing patterns of cells during waking tend to recur during sleep (e.g., (Pavlides and Winson, 1989, Wilson and McNaughton, 1994, Nadasdy et al., 1999, Dave and Margoliash, 2000, Louie and Wilson, 2001). Several neuronal changes during sleep have been related to learning. These include hippocampal sharp waves (Nadasdy et al., 1999), phasic pontine-waves (Datta, 2000), phase shift of hippocampal firing activity (Bjorness et al., 2005) and synaptic downscaling (Tononi and Cirelli, 2001). In addition, genes which are essential for synaptic plasticity and memory formation (Jones et al., 2001, Davis et al., 2003) are regulated during sleep (Ribeiro et al., 1999, Cirelli et al., 2004). In contrast to a direct involvement of sleep in memory consolidation, several studies suggest that sleep may facilitate memory consolidation by preventing waking interference. Motor activity, sensory input or additional learning occurring during waking can be sources of interference with the process of memory consolidation. Thus, there is evidence in humans that training of an additional task during the retention phase (Walker et al., 2003) as well as repeated testing of the same task (Mednick et al., 2002) can reverse a previously consolidated memory into a labile state, whereas restful waking can facilitate learning and provide similar benefits as sleep (Gottselig et al., 2004). During the SD, the mice were activated by introducing nesting material (tissues) and by other subtle stimulations. These motor and sensory stimuli could have interfered with memory consolidation.

All groups displayed a progressive reduction of object exploration during acquisition. Such habituation to objects during the acquisition phase has been shown previously in a similar paradigm in mice (Genoux et al., 2002). When the animals encountered the objects again in the test phase, it was evident that they all recognized the target objects as familiar because they did not increase the exploration of the familiar object (Fig. 2 A and B). Therefore, neither controls nor the SD groups had a memory consolidation problem. Despite intact memory consolidation, the 0-6 SD group treated the novel object as familiar, thus failing to recognize the novelty. This result suggests that the objects were remembered as a general category, indicating that mice were capable of learning in one trial an absolute class concept ‘object’ and to form a familiarity-based memory for other objects belonging to the same class. Therefore, in contrast to the deficit in object discrimination,

neither SD paradigm interfered with the formation of familiarity-based memory for a discrete object. In summary, the cognitive deficit induced by SD was related to a specific process involved in recognition memory.

It is unlikely that lack of motivation was responsible for the deficit in object discrimination observed in the early SD group, because the 24-h retention interval and the 18 and 12 h recovery from SD in the early and late SD groups, respectively, allowed sufficient time to recover from the sleep deprivation, as shown by Kopp et al. (Kopp et al., 2002) and supported by the time course of rest and activity after the SD (Fig. 3). Despite the larger loss of sleep due to the circadian timing of the SD in the 7-12 SD group, memory was impaired only in the 0-6 SD group. Therefore, it was a memory deficiency and not lack of motivation that was impaired by the early SD. This interpretation is supported by the slightly longer time spent exploring the objects upon test in this early SD group compared to the 7-12 SD group.

A stable memory for a whole scene requires knowledge of the specific features of objects, but also of their spatio-temporal relationships (Graham and Gaffan, 2005). Hence, inability to retrieve spatio-temporal information might have been responsible for the performance deficit induced by SD. One can hypothesize that the 0-6 h SD animals might have encoded the new scene encountered at test as a novel one, despite the presence of the (familiar) targets. To evaluate this possibility, we tested the ability of the mice to detect a spatial rearrangement of familiar objects. The mice failed to discriminate between the objects in the familiar and novel location 24 h later, precluding the assessment of SD effects. This incapacity is consistent with data showing that C57BL/6 mice and several rat strains (DA, Sprague-Dawley and Lister) tested in the object location task successfully discriminated between the familiar and novel object location after a delay interval of up to 6 h, while others (DBA/2 mice and Wistar rats) did not (Roullet et al., 1997, Dix and Aggleton, 1999, Ennaceur et al., 2005, Hotte et al., 2005, Lee et al., 2005). This failure in discrimination between the familiar and novel location of the objects can be related to either impaired memory for the object *per se* or inability to use spatial information after the relatively long delay of 24 h. Because the mice were able to detect object familiarity in the complex scene, it is likely that retrieval of spatial memory was hampered.

It is well known that sleep-wake or rest-activity behavior varies across 24 h in rodents. Thus, the OF1 strain exhibits higher levels of activity during the first half of the light period than in the second 6 h (Kopp et al., 2002). Consistent with this finding, the delayed SD induced a larger loss of rest than the immediate one, when it was compared to the amount of rest the animals obtained during the corresponding baseline interval. Despite this difference in loss of rest between the SD groups, it was the 7-12 SD group that showed no SD impairment on memory. It is well known that 6 h SD induce a prominent increase in SWA in NREM sleep during recovery (e.g., (Tobler, 2005). The significant increase in rest consolidation during recovery after both SD paradigms could be reflecting an increase in sleep intensity (Fig. 4). A consolidation of rest occurred also after the acquisition phase in the mice that were left undisturbed. This finding may be a homeostatic response to sleep loss during the acquisition phase as well as a consequence of the learning paradigm. The rest consolidation following upon learning supports the notion

that early sleep (or rest) may provide optimal conditions for memory consolidation.

Stress may be a confounding factor of SD procedures (Horne and McGrath, 1984). Both acute stress and a rise in the levels of stress hormones can modify memory formation and processing at retrieval, depending on the stressor and the magnitude of hormone increase (for review (Vanderwolf, 1969, Kim et al., 2005). In our study, exposure to novel objects during the acquisition phase induced a rise of corticosterone, which gradually returned to baseline during the following 6 h, even when animals were sleep deprived (Fig. 5). Importantly, despite slightly higher plasma levels of corticosterone in animals sleep-deprived immediately after acquisition compared to mice that were left undisturbed, both levels did not differ from undisturbed time-matched controls. Moreover, plasma levels of ACTH and corticosterone in the sleep-deprived animals were much lower than those of the immobilized mice. We conclude that it is unlikely that stress contributed to the memory deficit induced by SD. Moreover, the memory deficit was selective, since familiarity processing was intact. Hence, any difference in performance between the groups can be attributed to the early intervention, the only factor varying consistently across groups.

In conclusion, the loss of sleep or the additional activities of the mice during the SD impaired recognition memory retrieval, when they were incurred immediately after acquisition, whereas the delayed SD that had allowed 6 h of undisturbed sleep had no such effect. Neither SD schedule impaired object familiarity processing, suggesting a selectivity of the cognitive impairment induced by the intervention.

Acknowledgment

We thank members of the group for their help during the SD. This work was supported by the Swiss National Science Foundation grants nr. 3100-053005.97/2 and 3100A0-100567/1 and EU grant STREP-518189.

References

Bjorness, T. E., Riley, B. T., Tysor, M. K., & Poe, G. R. (2005). REM restriction persistently alters strategy used to solve a spatial task. *Learn Mem*, 12**,** 352-359.

Bour, A., Little, S., Dodart, J. C., Kelche, C., & Mathis, C. (2004). A secreted form of the beta-amyloid precursor protein (sAPP695) improves spatial recognition memory in OF1 mice. *Neurobiol Learn Mem*, 81**,** 27-38.

Chaudhury, D., & Colwell, C. S. (2002). Circadian modulation of learning and memory in fear-conditioned mice. *Behav Brain Res*, 133**,** 95-108.

Cirelli, C., Gutierrez, C. M., & Tononi, G. (2004). Extensive and divergent effects of sleep and wakefulness on brain gene expression. *Neuron*, 41**,** 35-43.

Coenen, A. (2005). Where is the classic interference theory for sleep and memory? *Behavioral and Brain Sciences*, 28**,** 67-68.

Datta, S. (2000). Avoidance task training potentiates phasic pontine-wave density in the rat: a mechanism for sleep-dependent plasticity. *J. Neurosci.*, 20**,** 8607-8613.

Dave, A. S., & Margoliash, D. (2000). Song replay during sleep and computational rules for sensorimotor vocal learning. *Science*, 290**,** 812-816.

Davis, S., Bozon, B., & Laroche, S. (2003). How necessary is the activation of the immediate early gene zif268 in synaptic plasticity and learning? *Behav Brain Res*, 142**,** 17-30.

Dere, E., Silva, M. A. D., & Huston, J. P. (2004). Higher order memories for objects encountered in different spatio-temporaral contexts in mice: Evidence for episodic memory. *Reviews in the Neurosciences*, 15**,** 231-240.

Dix, S. L., & Aggleton, J. P. (1999). Extending the spontaneous preference test of recognition: evidence of object-location and object-context recognition. *Behav Brain Res*, 99**,** 191-200.
Dodart, J. C., Mathis, C., & Ungerer, A. (1997). Scopolamine-induced deficits in a two-trial object recognition task in mice. *Neuroreport*, 8**,** 1173-1178.
Doyon, J., Carrier, J., Simard, A., Tahar, A. H., Morin, A., Benali, H., & Ungerleider, L. G. (2005). Motor memory: Consolidation-based enhancement effect revisited. *Behavioral and Brain Sciences*, 28**,** 68-69.
Ennaceur, A., & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behav. Brain Res.*, 31**,** 47-59.
Ennaceur, A., & Meliani, K. (1992). A new one-trial test for neurobiological studies of memory in rats. III. Spatial vs. non-spatial working memory. *Behav. Brain Res.*, 51**,** 83-92.
Ennaceur, A., Michalikova, S., Bradford, A., & Ahmed, S. (2005). Detailed analysis of the behavior of Lister and Wistar rats in anxiety, object recognition and object location tasks. *Behav Brain Res*, 159**,** 247-266.
Gaffan, E. A., Healey, A. N., & Eacott, M. J. (2004). Objects and positions in visual scenes: effects of perirhinal and postrhinal cortex lesions in the rat. *Behav. Neurosci.*, 118**,** 992-1010.
Genoux, D., Haditsch, U., Knobloch, M., Michalon, A., Storm, D., & Mansuy, I. M. (2002). Protein phosphatase 1 is a molecular constraint on learning and memory. *Nature*, 418**,** 970-975.
Gottselig, J. M., Hofer-Tinguely, G., Borbély, A. A., Regel, S. J., Landolt, H. P., Retey, J. V., & Achermann, P. (2004). Sleep and rest facilitate auditory learning. *Neuroscience*, 127**,** 557-561.
Graves, L. A., Heller, E. A., Pack, A. I., & Abel, T. (2003). Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. *Learn. Mem.*, 10**,** 168-176.
Hirase, H., Leinekugel, X., Czurko, A., Csicsvari, J., & Buzsaki, G. (2001). Firing rates of hippocampal neurons are preserved during subsequent sleep episodes and modified by novel awake experience. *Proc Natl Acad Sci U S A*, 98**,** 9386-9390.
Horne, J. A., & McGrath, M. J. (1984). The consolidation hypothesis for REM sleep function: stress and other confounding factors--a review. *Biol Psychol*, 18**,** 165-184.
Hotte, M., Naudon, L., & Jay, T. M. (2005). Modulation of recognition and temporal order memory retrieval by dopamine D(1) receptor in rats. *Neurobiol Learn Mem*, 84**,** 85-92.
Jones, M. W., Errington, M. L., French, P. J., Fine, A., Bliss, T. V., Garel, S., Charnay, P., Bozon, B., Laroche, S., & Davis, S. (2001). A requirement for the immediate early gene Zif268 in the expression of late LTP and long-term memories. *Nat Neurosci*, 4**,** 289-296.
Kim, J. J., & Diamond, D. M. (2002). The stressed hippocampus, synaptic plasticity and lost memories. *Nat. Rev. Neurosci.*, 3**,** 453-462.
Kopp, C., Petit, J. M., Magistretti, P., Borbély, A. A., & Tobler, I. (2002). Comparison of the effects of modafinil and sleep deprivation on sleep and cortical EEG spectra in mice. *Neuropharmacology*, 43**,** 110-118.
Korman, M., Flash, T., & Karni, A. (2005). Resistance to interference and the emergence of delayed gains in newly acquired procedural memories: Synaptic and system consolidation? *Behavioral and Brain Sciences*, 28**,** 74-75.
Lee, I., Hunsaker, M. R., & Kesner, R. P. (2005). The role of hippocampal subregions in detecting spatial novelty. *Behav Neurosci*, 119**,** 145-153.
Louie, K., & Wilson, M. A. (2001). Temporally structured replay of awake hippocampal ensemble activity during rapid eye movement sleep. *Neuron*, 29**,** 145-156.
Mednick, S. C., Nakayama, K., Cantero, J. L., Atienza, M., Levin, A. A., Pathak, N., & Stickgold, R. (2002). The restorative effect of naps on perceptual deterioration. *Nat Neurosci*, 5**,** 677-681.
Morris, R. G. (2001). Episodic-like memory in animals: psychological criteria, neural mechanisms and the value of episodic-like tasks to investigate animal models of neurodegenerative disease. *Philos Trans R Soc Lond B Biol Sci*, 356**,** 1453-1465.
Nadasdy, Z., Hirase, H., Czurko, A., Csicsvari, J., & Buzsaki, G. (1999). Replay and time compression of recurring spike sequences in the hippocampus. *J Neurosci*, 19**,** 9497-9507.
Palchykova, S., Crestani, F., Meerlo, P., & Tobler, I. (2005). Sleep deprivation and daily torpor impair object recognition in Djungarian hamsters. *Physiology & Behavior*, in press.
Pavlides, C., & Winson, J. (1989). Influences of hippocampal place cell firing in the awake state on the activity of these cells during subsequent sleep episodes. *J Neurosci*, 9**,** 2907-2918.
Pearlman, C. (1973). REM sleep deprivation impairs latent extinction in rats. *Physiol Behav*, 11**,** 233-237.
Poe, G. R., Nitz, D. A., McNaughton, B. L., & Barnes, C. A. (2000). Experience-dependent phase-reversal of hippocampal neuron firing during REM sleep. *Brain Res*, 855**,** 176-180.

Ribeiro, S., Goyal, V., Mello, C. V., & Pavlides, C. (1999). Brain gene expression during REM sleep depends on prior waking experience. *Learn Mem*, 6**,** 500-508.
Roullet, P., Mele, A., & Ammassari-Teule, M. (1997). Ibotenic lesions of the nucleus accumbens promote reactivity to spatial novelty in nonreactive DBA mice: implications for neural mechanisms subserving spatial information encoding. *Behav Neurosci*, 111**,** 976-984.
Schredl, M. (2005). REM sleep, dreaming, and procedural memory. *Behavioral and Brain Sciences*, 28**,** 80-81.
Siegel, J. M. (2005). The incredible, shrinking sleep-learning connection. *Behavioral and Brain Sciences*, 28**,** 82-83.
Smith, C., & Butler, S. (1982). Paradoxical sleep at selective times following training is necessary for learning. *Physiol Behav*, 29**,** 469-473.
Smith, C., & Rose, G. M. (1996). Evidence for a paradoxical sleep window for place learning in the Morris water maze. *Physiol. Behav.*, 59**,** 93-97.
Smith, C., Tenn, C., & Annett, R. (1991). Some biochemical and behavioural aspects of the paradoxical sleep window. *Can J Psychol*, 45**,** 115-124.
Smith, C. T., Conway, J. M., & Rose, G. M. (1998). Brief paradoxical sleep deprivation impairs reference, but not working, memory in the radial arm maze task. *Neurobiol. Learn. Mem.*, 69**,** 211-217.
Stickgold, R. (2005). Sleep-dependent memory consolidation. *Nature*, 437**,** 1272-1278.
Tobler, I. (2005). Phylogeny of Sleep Regulation. *In* M. H. Kryger, T. Roth, & W. C. Dement (Eds.), *Principles and practice of sleep medicine* (pp. 72-81). Philadelphia, PA: Elsevier Saunders.
Tobler, I., Deboer, T., & Fischer, M. (1997). Sleep and sleep regulation in normal and prion protein-deficient mice. *J. Neurosci.*, 17**,** 1869-1879.
Tobler, I., Gaus, S. E., Deboer, T., Achermann, P., Fischer, M., Rulicke, T., Moser, M., Oesch, B., McBride, P. A., & Manson, J. C. (1996). Altered circadian activity rhythms and sleep in mice devoid of prion protein. *Nature*, 380**,** 639-642.
Tononi, G., & Cirelli, C. (2001). Some considerations on sleep and neural plasticity. *Arch. Ital. Biol.*, 139**,** 221-241.
Vertes, R. P. (2004). Memory consolidation in sleep; dream or reality. *Neuron*, 44**,** 135-148.
Vertes, R. P. (2005). Sleep is for rest, waking consciousness is for learning and memory - of any kind. *Behavioral and Brain Sciences*, 28**,** 86-87.
Vertes, R. P., & Siegel, J. M. (2005). Time for the sleep community to take a critical look at the purported role of sleep in memory processing. *Sleep*, 28**,** 1228-1229; discussion 1230-1233.
Walker, M. P. (2004). Issues surrounding sleep-dependent memory consolidation and plasticity. *Cell Mol Life Sci*, 61**,** 3009-3015.
Walker, M. P., Brakefield, T., Hobson, J. A., & Stickgold, R. (2003). Dissociable stages of human memory consolidation and reconsolidation. *Nature*, 425**,** 616-620.
Wilson, M. A., & McNaughton, B. L. (1994). Reactivation of hippocampal ensemble memories during sleep. *Science*, 265**,** 676-679.
Wolf, O. T. (2003). HPA axis and memory. *Best Pract Res Clin Endocrinol Metab*, 17**,** 287-299.

GENERAL DISCUSSION

Sleep is a ubiquitous phenomenon which is regulated by two independent processes, a homeostatic process and a circadian process (Borbély and Achermann, 2005). EEG SWA in NREM sleep represents a marker of sleep intensity (Borbély, 1982). The investigation of sleep and its regulation under different physiological conditions in a variety of animals contributes to the understanding of the mechanisms underlying sleep regulation. They might reveal the function(s) of sleep, particularly its role in learning and memory consolidation. The present thesis is focused on the homeostatic regulation of sleep after episodes of daily torpor in the Djungarian hamster, on the effects of seasonality on sleep and on the involvement of sleep in memory consolidation. The two-process model of sleep regulation served as a framework.

The aim of the first paper was to investigate whether sleep after daily torpor is homeostatically regulated. This was accomplished by manipulating slow-waves immediately after emergence from a torpor episode in the Djungarian hamster. It has been shown previously in Djungarian hamsters (Deboer and Tobler, 1994) and ground squirrels that daily torpor or a hibernation bout lasting 1.7-12.7 days (Daan et al., 1991, Trachsel et al., 1991) is followed by an initial high value of SWA in NREM sleep and a subsequent decline. It has been suggested that sleep after daily torpor is homeostatically regulated (Deboer and Tobler, 1994, Deboer and Tobler, 2000). SD performed immediately after termination of a daily torpor episode led to an additional increase of SWA in NREM sleep compared to SD alone (Deboer and Tobler, 2000). However, similar experiments in ground squirrels resulted in a disappearance of SWA when SD was performed after a hibernation bout (Larkin and Heller, 1998, Strijkstra and Daan, 1998). However, the squirrels had obtained approximately 20-30% of sleep during the SD that might have been sufficient to compensate for the sleep debt. In hamsters, partial NREM sleep deprivation allowing 20-30% of sleep was able to maintain sleep pressure at a high level and did not result in a lowering or disappearance of SWA. Instead, the increase in SWA in NREM sleep after torpor followed by partial NREM sleep deprivation was similar to the increase after 4 h SD followed by the same duration of partial NREM sleep deprivation (Palchykova et al., 2002). The data confirm the hypothesis that animals incur a sleep debt during hypothermia and that sleep after daily torpor is homeostatically regulated. Further studies are needed to evaluate whether similar mechanisms are involved in regulation of sleep after inducing torpor artificially in other species (e.g. (Zhang et al., 2006)). Specifically, they might answer the questions whether pharmacologically induced torpor in other rodents is also associated with accumulation of a sleep deficit. Results of such experiments might be implicated in induction of hypothermia in humans for medical purposes.

It has been shown previously in several species that SD increases regional differences in EEG SWA in NREM sleep during recovery. This was the case in humans, rats and mice (Cajochen et al., 1999, Schwierin et al., 1999, Huber et al., 2000b). Therefore, an investigation of regional differences in sleep in hamsters was the second aim of this paper. SD of 4 h induced a frontal predominance in SWA in NREM sleep. Observed regional differences

might have use-dependent character due to higher working load in the frontal region of the brain. Surprisingly, also daily torpor was followed by a prolonged predominance of SWA in NREM sleep in the frontal derivation (Palchykova et al., 2002). Moreover, torpor-induced regional differences were large compared to SD-induced differences. Further studies should be performed to identify the mechanisms underlying regional differences in the EEG after daily torpor. It has to be clarified whether synapses that were insufficiently used during torpor must be stimulated (Krueger and Obál, 1993, Kavanau, 1999) or up-scaled during euthermic sleep (Tononi and Cirelli, 2006).

Homeostatic properties of sleep were further investigated by examining sleep in hamsters exhibiting winter or summer physiology despite continuous winter-like environmental conditions. Although day-length appeared to be the primary environmental cue that the Djungarian hamster uses to initiate seasonally appropriate physiological and behavioral changes, there seems to be an endogenous component contributing to the seasonal changes in sleep. A redistribution of sleep-wake behavior had been shown previously in hamsters adapted to a long photoperiod after prolonged exposure to a short photoperiod (Deboer et al., 2000). However, the question remained whether the changes were induced by the photoperiod itself or by an endogenous component. Our data showed for the first time that the polyphasic sleep-wake pattern was differently distributed in hamsters recorded in different physiological states: typical for winter or for summer, although the total amount of vigilance states remained unchanged (Palchykova et al., 2003). Thus, hamsters in the summer physiological state sleep less but more intensively during the dark period compared to their sleep in winter. The changes in SWA in NREM sleep reflected the changes in the amount of sleep within the light or dark period. In winter, when sleep was evenly distributed between the light and dark period, SWA showed only minimal light-dark amplitude. The sleep-wake redistribution observed in animals in the winter and summer physiological state was reflected also in the frequency and duration of the vigilance states episode. These data are in accordance with the two-process model of sleep regulation predicting that the homeostatic process S, quantified by SWA, reflects the previous sleep-wake history. The present results show that the animal's physiology, including sleep, returns to its summer characteristics despite the continuous exposure to a short photoperiod and low ambient temperature.

There is evidence that sleep might be involved in memory consolidation (Stickgold, 2005, Walker, 2005). However, nothing is known about the involvement of sleep in consolidation of recognition memory in rodents, and specifically about the relationship between sleep and memory in the Djungarian hamster. In the third paper the effects of SD and daily torpor on recognition memory consolidation in an object recognition task were investigated. A post-learning SD led to a recency memory detriment in a simple two-object choice situation 24 h after encoding, while both the formation of a familiarity-based memory for the object and detection and processing of object novelty were not altered by the prior loss of sleep (Palchykova et al., 2006a). The negative effects of SD on object recognition increased with the level of difficulty of the task. In a complex scene task, sleep-deprived hamsters displayed a deficit in familiarity-based memory at retrieval. Sleep deprived animals failed to discriminate between two familiar

identical objects on the basis of the familiarity or novelty of their spatial location. Thus, depending on the difficulty of the discrimination task, a prolongation of wakefulness hampers recency memory for discrete objects and retrieval of scene memory, while sparing object familiarity-based recognition and novelty processing.

The occurrence of daily torpor during retention was associated with a selective impairment in scene memory retrieval in the complex scene task (Palchykova et al., 2006a). However, familiarity- and recency-based recognition and object novelty processing stayed intact. The scene memory retrieval deficit seen in torpor hamsters was analogous to that induced by SD, suggesting the existence of detrimental factors common to both conditions. These factors still have to be identified. The occurrence of daily torpor later in the retention period contributed to a less pronounced cognitive impairment after torpor compared to SD.

Our data provide additional evidence that a transient sleep deficit during an early or late time window of the retention period has no effect on familiarity and novelty processing but impairs recency and spatial scene memory retrieval of objects, depending on the difficulty of the task. The sleep deficit would not interfere with the formation of object memories but rather with cognitive abilities, which are essential for their retrieval. Thus, our data provide evidence that sleep is important for retrieval of memory in the Djungarian hamster.

In the fourth paper we further investigated whether timing of sleep is important for consolidation of object recognition memory in mice. A period of SD following immediately upon the acquisition phase of an object recognition task caused a significant retrieval detriment in a complex scene paradigm (Palchykova et al., 2006b). This deficit in object discrimination was restricted to the group of mice subjected to SD during the first 6 h following acquisition, since both the controls that were allowed to sleep as well as the mice subjected to a similar but delayed SD had no such deficit. All in all the results confirm that the timing of sleep is crucial for its beneficial effect on memory consolidation and retrieval. A familiarity-based memory for a discrete object was intact after both SDs, indicating that the cognitive deficit induced by SD was related to a specific process involved in recognition memory. Therefore, the loss of sleep or the additional activities during the SD impaired recognition memory retrieval, but only when they were incurred immediately after acquisition. It has to be clarified whether physiological processes taking place during sleep actively facilitate memory consolidation or whether sleep provides optical conditions of non-interference for consolidation. If the latter is the case then a period of quiet waking with a low sensory load must provide similar benefits as sleep.

The main manipulation that was used in our studies is sleep deprivation. Therefore, the method of SD was especially adapted to minimize stress. Our animals were provided with nesting material during SD and were not disturbed during eating and drinking. They were never directly touched by the observer. This was particularly important in the learning and memory experiments because data suggest that stress leads to memory impairment (Wolf, 2003, Lynch, 2004). To make sure that our experiments were not affected by potential SD-induced stress, plasma levels of the stress hormones were determined. The absence of differences between animals subjected to

the learning paradigm followed by SD and undisturbed home-cage controls suggested that stress was not the main contributing factor neither in hamsters nor in mice.

The findings presented in this thesis support the hypothesis that sleep after daily torpor is homeostatically regulated and provide evidence for the endogenous regulation of sleep in seasonal animals and show a critical involvement of sleep in recognition memory consolidation in rodents.

REFERENCES

Achermann, P. and Borbély, A. A., 2003. Mathematical models of sleep regulation. Front Biosci. 8, s683-693.

Aeschbach, D., Dijk, D. J. and Borbely, A. A., 1997. Dynamics of EEG spindle frequency activity during extended sleep in humans: relationship to slow-wave activity and time of day. Brain Res. 748, 131-136.

Ambrosini, M. V., Langella, M., Gironi Carnevale, U. A. and Giuditta, A., 1992. The sequential hypothesis of sleep function. III. The structure of postacquisition sleep in learning and nonlearning rats. Physiol Behav. 51, 217-226.

Amzica, F. and Massimini, M., 2002. Glial and neuronal interactions during slow wave and paroxysmal activities in the neocortex. Cereb Cortex. 12, 1101-1113.

Amzica, F., Massimini, M. and Manfridi, A., 2002. Spatial buffering during slow and paroxysmal sleep oscillations in cortical networks of glial cells in vivo. J Neurosci. 22, 1042-1053.

Amzica, F. and Steriade, M., 1995. Short- and long-range neuronal synchronization of the slow (< 1 Hz) cortical oscillation. J Neurophysiol. 73, 20-38.

Amzica, F. and Steriade, M., 1998. Electrophysiological correlates of sleep delta waves. Electroencephalogr Clin Neurophysiol. 107, 69-83.

Arendt, J. and Skene, D. J., 2005. Melatonin as a chronobiotic. Sleep Med Rev. 9, 25-39.

Baker, K. B. and Kim, J. J., 2002. Effects of stress and hippocampal NMDA receptor antagonism on recognition memory in rats. Learn Mem. 9, 58-65.

Bal, T., von Krosigk, M. and McCormick, D. A., 1995. Role of the ferret perigeniculate nucleus in the generation of synchronized oscillations in vitro. J Physiol. 483 (Pt 3), 665-685.

Barre, V. and Petterrousseaux, A., 1988. Seasonal-Variations in Sleep-Wake Cycle in *Microcebus murinus*. Primates. 29, 53-64.

Bazhenov, M., Timofeev, I., Steriade, M. and Sejnowski, T. J., 2002. Model of thalamocortical slow-wave sleep oscillations and transitions to activated States. J Neurosci. 22, 8691-8704.

Beaulieu, I. and Godbout, R., 2000. Spatial learning on the Morris Water Maze Test after a short-term paradoxical sleep deprivation in the rat. Brain Cogn. 43, 27-31.

Beersma, D. G., Dijk, D. J., Blok, C. G. and Everhardus, I., 1990. REM sleep deprivation during 5 hours leads to an immediate REM sleep rebound and to suppression of non-REM sleep intensity. Electroencephalogr Clin Neurophysiol. 76, 114-122.

Benington, J. H. and Heller, H. C., 1994. REM-sleep timing is controlled homeostatically by accumulation of REM-sleep propensity in non-REM sleep. Am J Physiol. 266, R1992-2000.

Benington, J. H. and Heller, H. C., 1995. Restoration of brain energy metabolism as the function of sleep. Prog Neurobiol. 45, 347-360.

Berger, R. J., 1975. Bioenergetic functions of sleep and activity rhythms and their possible relevance to aging. Fed Proc. 34, 97-102.

Berger, R. J., 1984. Slow wave sleep, shallow torpor and hibernation: homologous states of diminished metabolism and body temperature. Biol Psychol. 19, 305-326.

Berriel Diaz, M., Lange, M., Heldmaier, G. and Klingenspor, M., 2004. Depression of transcription and translation during daily torpor in the Djungarian hamster (Phodopus sungorus). J Comp Physiol [B]. 174, 495-502.

Bittman, E. L., 1978. Hamster refractoriness: the role of insensitivity of pineal target tissues. Science. 202, 648-650.

Bjorness, T. E., Riley, B. T., Tysor, M. K. and Poe, G. R., 2005. REM restriction persistently alters strategy used to solve a spatial task. Learn Mem. 12, 352-359.

Blackstone, E., Morrison, M. and Roth, M. B., 2005. H_2S Induces a Suspended Animation-Like State in Mice. Science. 308, 518.

Bland, B. H. and Colom, L. V., 1993. Extrinsic and intrinsic properties underlying oscillation and synchrony in limbic cortex. Prog Neurobiol. 41, 157-208.

Bockers, T. M., Bockmann, J., Salem, A., Niklowitz, P., Lerchl, A., Huppertz, M., Wittkowski, W. and Kreutz, M. R., 1997. Initial expression of the common alpha-chain in hypophyseal pars tuberalis-specific cells in spontaneous recrudescent hamsters. Endocrinology. 138, 4101-4108.

Borbély, A. A., 1982. A two process model of sleep regulation. Hum Neurobiol. 1, 195-204.

Borbély, A. A. and Achermann, P., 2005. Sleep Homeostasis and Models of Sleep Regulation. In: Kryger, M. H. et al. (Eds.), Principles and practice of sleep medicine. Elsevier Saunders, Philadelphia, PA, pp. 405-417.
Borbély, A. A., Baumann, F., Brandeis, D., Strauch, I. and Lehmann, D., 1981. Sleep deprivation: effect on sleep stages and EEG power density in man. Electroencephalogr Clin Neurophysiol. 51, 483-495.
Borbély, A. A. and Neuhaus, H. U., 1978. Daily pattern of sleep, motor activity and feeding in rat: effects of regular and gradually extended photoperiods. J Comp Physiol. 124, 1-14.
Borbély, A. A., Tobler, I. and Hanagasioglu, M., 1984. Effect of sleep deprivation on sleep and EEG power spectra in the rat. Behav Brain Res. 14, 171-182.
Borisyuk, R. and Hoppensteadt, F., 1999. Oscillatory models of the hippocampus: a study of spatio-temporal patterns of neural activity. Biol Cybern. 81, 359-371.
Bowman, R. E., 2005. Stress-induced changes in spatial memory are sexually differentiated and vary across the lifespan. J Neuroendocrinol. 17, 526-535.
Brazhnik, E. S. and Fox, S. E., 1999. Action potentials and relations to the theta rhythm of medial septal neurons in vivo. Exp Brain Res. 127, 244-258.
Buzsaki, G., 2002. Theta oscillations in the hippocampus. Neuron. 33, 325-340.
Cajochen, C., Foy, R. and Dijk, D.-J., 1999. Frontal predominance of a relative increase in sleep delta and theta EEG activity after sleep loss in humans. Sleep Res Online. 2, 65-69.
Celesia, G. G. and Jasper, H. H., 1966. Acetylcholine released from cerebral cortex in relation to state of activation. Neurology. 16, 1053-1063.
Cirelli, C., 2002. How sleep deprivation affects gene expression in the brain: a review of recent findings. J Appl Physiol. 92, 394-400.
Cirelli, C., Gutierrez, C. M. and Tononi, G., 2004. Extensive and divergent effects of sleep and wakefulness on brain gene expression. Neuron. 41, 35-43.
Cirelli, C., LaVaute, T. M. and Tononi, G., 2005. Sleep and wakefulness modulate gene expression in Drosophila. J Neurochem. 94, 1411-1419.
Cirelli, C. and Tononi, G., 1998. Differences in gene expression between sleep and waking as revealed by mRNA differential display. Brain Res Mol Brain Res. 56, 293-305.
Connors, B. W., Gutnick, M. J. and Prince, D. A., 1982. Electrophysiological properties of neocortical neurons in vitro. J Neurophysiol. 48, 1302-1320.
Conrad, C. D., Grote, K. A., Hobbs, R. J. and Ferayorni, A., 2003. Sex differences in spatial and non-spatial Y-maze performance after chronic stress. Neurobiol Learn Mem. 79, 32-40.
Conrad, C. D., Jackson, J. L., Wieczorek, L., Baran, S. E., Harman, J. S., Wright, R. L. and Korol, D. L., 2004. Acute stress impairs spatial memory in male but not female rats: influence of estrous cycle. Pharmacol Biochem Behav. 78, 569-579.
Contreras, D., Curro Dossi, R. and Steriade, M., 1992. Bursting and tonic discharges in two classes of reticular thalamic neurons. J Neurophysiol. 68, 973-977.
Contreras, D., Destexhe, A., Sejnowski, T. J. and Steriade, M., 1996a. Control of spatiotemporal coherence of a thalamic oscillation by corticothalamic feedback. Science. 274, 771-774.
Contreras, D., Destexhe, A., Sejnowski, T. J. and Steriade, M., 1997. Spatiotemporal patterns of spindle oscillations in cortex and thalamus. J Neurosci. 17, 1179-1196.
Contreras, D., Timofeev, I. and Steriade, M., 1996b. Mechanisms of long-lasting hyperpolarizations underlying slow sleep oscillations in cat corticothalamic networks. J Physiol. 494 (Pt 1), 251-264.
Crick, F. and Mitchison, G., 1983. The function of dream sleep. Nature. 304, 111-114.
Daan, S., Barnes, B. M. and Strijkstra, A. M., 1991. Warming up for sleep? - ground squirrels sleep during arousal from hibernation. Neurosci Lett. 128, 265-268.
Danguir, J. and Nicolaidis, S., 1976. Impairments of learned aversion acquisition following paraodixcal sleep deprivation in the rat. Physiol Behav. 17, 489-492.
Datta, S., 1997. Cellular basis of pontine ponto-geniculo-occipital wave generation and modulation. Cell Mol Neurobiol. 17, 341-365.
Datta, S., 2000. Avoidance task training potentiates phasic pontine-wave density in the rat: a mechanism for sleep-dependent plasticity. J Neurosci. 20, 8607-8613.
Datta, S., Mavanji, V., Ulloor, J. and Patterson, E. H., 2004. Activation of phasic pontine-wave generator prevents rapid eye movement sleep deprivation-induced learning

impairment in the rat: a mechanism for sleep-dependent plasticity. J Neurosci. 24, 1416-1427.
Dave, A. S. and Margoliash, D., 2000. Song replay during sleep and computational rules for sensorimotor vocal learning. Science. 290, 812-816.
Davis, S., Bozon, B. and Laroche, S., 2003. How necessary is the activation of the immediate early gene zif268 in synaptic plasticity and learning? Behav Brain Res. 142, 17-30.
Dawood, M. Y., Lumley, L. A., Robison, C. L., Saviolakis, G. A. and Meyerhoff, J. L., 2004. Accelerated Barnes maze test in mice for assessment of stress effects on memory. Ann N Y Acad Sci. 1032, 304-307.
Deboer, T., Franken, P. and Tobler, I., 1994. Sleep and cortical temperature in the Djungarian hamster under baseline conditions and after sleep deprivation. J Comp Physiol [A]. 174, 145-155.
Deboer, T. and Tobler, I., 1994. Sleep EEG after daily torpor in the Djungarian hamster: similarity to the effects of sleep deprivation. Neurosci Lett. 166, 35-38.
Deboer, T. and Tobler, I., 1996a. Natural hypothermia and sleep deprivation: common effects on recovery sleep in the Djungarian hamster. Am J Physiol. 271, R1364-R1371.
Deboer, T. and Tobler, I., 1996b. Shortening of the photoperiod affects sleep distribution, EEG and cortical temperature in the Djungarian hamster. J Comp Physiol [A]. 179, 483-492.
Deboer, T. and Tobler, I., 1997. Vigilance state episodes and cortical temperature in the Djungarian hamster: the influence of photoperiod and ambient temperature. Pflugers Arch. 433, 230-237.
Deboer, T. and Tobler, I., 2000. Slow waves in the sleep electroencephalogram after daily torpor are homeostatically regulated. Neuroreport. 11, 881-885.
Deboer, T. and Tobler, I., 2003. Sleep regulation in the Djungarian hamster: comparison of the dynamics leading to the slow-wave activity increase after sleep deprivation and daily torpor. Sleep. 26, 567-572.
Deboer, T., Vyazovskiy, V. V. and Tobler, I., 2000. Long photoperiod restores the 24-h rhythm of sleep and EEG slow-wave activity in the Djungarian hamster (Phodopus sungorus). J Biol Rhythms. 15, 429-436.
Denham, M. J. and Borisyuk, R. M., 2000. A model of theta rhythm production in the septal-hippocampal system and its modulation by ascending brain stem pathways. Hippocampus. 10, 698-716.
Dijk, D. J., Beersma, D. G. M. and Daan, S., 1987. EEG power density during nap sleep: reflection of an hourglass measuring the duration of prior wakefulness. J Biol Rhythms. 2, 207-219.
Dijk, D. J. and Daan, S., 1989. Sleep EEG spectral analysis in a diurnal rodent: *Eutamias sibiricus*. J Comp Physiol [A]. 165, 205-215.
Dossi, R. C., Nunez, A. and Steriade, M., 1992. Electrophysiology of a slow (0.5-4 Hz) intrinsic oscillation of cat thalamocortical neurones in vivo. J Physiol. 447, 215-234.
Dudai, Y., 2004. The neurobiology of consolidations, or, how stable is the engram? Annu Rev Psychol. 55, 51-86.
Dudai, Y. and Eisenberg, M., 2004. Rites of passage of the engram: reconsolidation and the lingering consolidation hypothesis. Neuron. 44, 93-100.
Endo, T., Roth, C., Landolt, H. P., Werth, E., Aeschbach, D., Achermann, P. and Borbely, A. A., 1998. Selective REM sleep deprivation in humans: effects on sleep and sleep EEG. Am J Physiol. 274, R1186-1194.
Endo, T., Schwierin, B., Borbely, A. A. and Tobler, I., 1997a. Selective and total sleep deprivation: effect on the sleep EEG in the rat. Psychiatry Res. 66, 97-110.
Endo, T., Schwierin, B., Borbély, A. A. and Tobler, I., 1997b. Selective and total sleep deprivation: effect on the sleep EEG in the rat. Psychiatry Res. 66, 97-110.
Figala, J., Hoffmann, K. and Goldau, G., 1973. Annual Cycle in Djungarian Hamster *Phodopus Sungorus* Pallas. Oecologia. 12, 89-118.
Finelli, L. A., Baumann, H., Borbely, A. A. and Achermann, P., 2000. Dual electroencephalogram markers of human sleep homeostasis: correlation between theta activity in waking and slow-wave activity in sleep. Neuroscience. 101, 523-529.
Finelli, L. A., Borbely, A. A. and Achermann, P., 2001. Functional topography of the human nonREM sleep electroencephalogram. Eur J Neurosci. 13, 2282-2290.
Fishbein, W., Kastaniotis, C. and Chattman, D., 1974. Paradoxical sleep: prolonged augmentation following learning. Brain Res. 79, 61-75.

Franken, P., 2002. Long-term vs. short-term processes regulating REM sleep. J Sleep Res. 11, 17-28.
Franken, P., Dijk, D. J., Tobler, I. and Borbely, A. A., 1991. Sleep deprivation in rats: effects on EEG power spectra, vigilance states, and cortical temperature. Am J Physiol. 261, R198-208.
Franken, P., Tobler, I. and Borbely, A. A., 1995. Varying photoperiod in the laboratory rat: profound effect on 24-h sleep pattern but no effect on sleep homeostasis. Am J Physiol. 269, R691-R701.
Freeman, D. A. and Zucker, I., 2001. Refractoriness to melatonin occurs independently at multiple brain sites in Siberian hamsters. Proc Natl Acad Sci U S A. 98, 6447-6452.
Fuentealba, P., Crochet, S., Timofeev, I., Bazhenov, M., Sejnowski, T. J. and Steriade, M., 2004a. Experimental evidence and modeling studies support a synchronizing role for electrical coupling in the cat thalamic reticular neurons in vivo. Eur J Neurosci. 20, 111-119.
Fuentealba, P., Timofeev, I., Bazhenov, M., Sejnowski, T. J. and Steriade, M., 2005. Membrane bistability in thalamic reticular neurons during spindle oscillations. J Neurophysiol. 93, 294-304.
Fuentealba, P., Timofeev, I. and Steriade, M., 2004b. Prolonged hyperpolarizing potentials precede spindle oscillations in the thalamic reticular nucleus. Proc Natl Acad Sci U S A. 101, 9816-9821.
Garcia, A., Marti, O., Valles, A., Dal-Zotto, S. and Armario, A., 2000. Recovery of the hypothalamic-pituitary-adrenal response to stress. Effect of stress intensity, stress duration and previous stress exposure. Neuroendocrinology. 72, 114-125.
Geiser, F., 2004. Metabolic rate and body temperature reduction during hibernation and daily torpor. Annu Rev Physiol. 66, 239-274.
Geiser, F. and Ruf, T., 1995. Hibernation versus Daily Torpor in Mammals and Birds: Physiological Variables and Classification of Torpor Patterns. Physiol Zool. 68, 935-966.
Giuditta, A., Ambrosini, M. V., Montagnese, P., Mandile, P., Cotugno, M., Grassi Zucconi, G. and Vescia, S., 1995. The sequential hypothesis of the function of sleep. Behav Brain Res. 69, 157-166.
Giuditta, A., Ambrosini, M. V., Scaroni, R., Chiurulla, C. and Sadile, A., 1985. Effect of sleep on cerebral DNA synthesized during shuttle-box avoidance training. Physiol Behav. 34, 769-778.
Glotzbach, S. F. and Heller, H. C., 1976. Central nervous regulation of body temperature during sleep. Science. 194, 537-539.
Graham, K. S. and Gaffan, D., 2005. The role of the medial temporal lobe in memory and perception: evidence from rats, nonhuman primates and humans. Q J Exp Psychol B. 58, 193-201.
Graves, L. A., Heller, E. A., Pack, A. I. and Abel, T., 2003. Sleep deprivation selectively impairs memory consolidation for contextual fear conditioning. Learn Mem. 10, 168-176.
Grigg, G. C., Beard, L. A. and Augee, M. L., 2004. The evolution of endothermy and its diversity in mammals and birds. Physiol Biochem Zool. 77, 982-997.
Gruart-Masso, A., Nadal-Alemany, R., Coll-Andreu, M., Portell-Cortes, I. and Marti-Nicolovius, M., 1995. Effects of pretraining paradoxical sleep deprivation upon two-way active avoidance. Behav Brain Res. 72, 181-183.
Guan, Z., Peng, X. and Fang, J., 2004. Sleep deprivation impairs spatial memory and decreases extracellular signal-regulated kinase phosphorylation in the hippocampus. Brain Res. 1018, 38-47.
Gutwein, B. M. and Fishbein, W., 1980. Paradoxical sleep and memory (I): Selective alterations following enriched and impoverished environmental rearing. Brain Res Bull. 5, 9-12.
Gwinner, E., 1989. Photoperiod as a modifying and limiting factor in the expression of avian circannual rhythms. J Biol Rhythms. 4, 237-250.
Gwinner, E., 2003. Circannual rhythms in birds. Curr Opin Neurobiol. 13, 770-778.
Hazlerigg, D. G., Gonzalez-Brito, A., Lawson, W., Hastings, M. H. and Morgan, P. J., 1993. Prolonged exposure to melatonin leads to time-dependent sensitization of adenylate cyclase and down-regulates melatonin receptors in pars tuberalis cells from ovine pituitary. Endocrinology. 132, 285-292.

Hazlerigg, D. G., Hastings, M. H. and Morgan, P. J., 1996. Production of a prolactin releasing factor by the ovine pars tuberalis. J Neuroendocrinol. 8, 489-492.
Heldmaier, G. and Ruf, T., 1992. Body temperature and metabolic rate during natural hypothermia in endotherms. J Comp Physiol [B]. 162, 696-706.
Heldmaier, G., Steinlechner, S., Ruf, T., Wiesinger, H. and Klingenspor, M., 1989. Photoperiod and thermoregulation in vertebrates: body temperature rhythms and thermogenic acclimation. J Biol Rhythms. 4, 251-265.
Heller, H. C., 1988. Sleep and hypometabolism. Can J Zool. 66, 61-69.
Heller, H. C., 2005. Temperature, Thermoregulation, and Sleep. In: Kryger, M. H. et al. (Eds.), Principles and practice of sleep medicine. Elsevier Saunders, Philadelphia, PA, pp. 292-304.
Hennevin, E. and Hars, B., 1987. Is increase in post-learning paradoxical sleep modified by cueing? Behav Brain Res. 24, 243-249.
Hoffman, K. L. and McNaughton, B. L., 2002. Coordinated reactivation of distributed memory traces in primate neocortex. Science. 297, 2070-2073.
Hoffmann, K., 1973. Influence of Photoperiod and Melatonin on Testis Size, Body-Weight, and Pelage Color in Djungarian Hamster (Phodopus-Sungorus). Journal of Comparative Physiology. 85, 267-282.
Holsheimer, J., Boer, J., Lopes da Silva, F. H. and van Rotterdam, A., 1982. The double dipole model of theta rhythm generation: simulation of laminar field potential profiles in dorsal hippocampus of the rat. Brain Res. 235, 31-50.
Huber, R., Deboer, T. and Tobler, I., 2000a. Effects of sleep deprivation on sleep and sleep EEG in three mouse strains: empirical data and simulations. Brain Res. 857, 8-19.
Huber, R., Deboer, T. and Tobler, I., 2000b. Topography of EEG dynamics after sleep deprivation in mice. J Neurophysiol. 84, 1888-1893.
Huber, R., Ghilardi, M. F., Massimini, M. and Tononi, G., 2004. Local sleep and learning. Nature. 430, 78-81. Epub 2004 Jun 2006.
Hui, Z., Guang-Yu, M., Chong-Tao, X., Quan, Y. and Xiao-Hu, X., 2005. Phenytoin reverses the chronic stress-induced impairment of memory consolidation for water maze training and depression of LTP in rat hippocampal CA1 region, but does not affect motor activity. Brain Res Cogn Brain Res. 24, 380-385.
Johnston, J. D., 2005. Measuring seasonal time within the circadian system: regulation of the suprachiasmatic nuclei by photoperiod. J Neuroendocrinol. 17, 459-465.
Johnston, J. D., Cagampang, F. R., Stirland, J. A., Carr, A. J., White, M. R., Davis, J. R. and Loudon, A. S., 2003. Evidence for an endogenous per1- and ICER-independent seasonal timer in the hamster pituitary gland. Faseb J. 17, 810-815.
Jones, B. I., 2005. Basic Mechanisms of Sleep-Wake States. In: Kryger, M. H. et al. (Eds.), Principles and practice of sleep medicine. Elsevier Saunders, Philadelphia, PA, pp. 136-153.
Jouvet, M., 1969. Biogenic amines and the states of sleep. Science. 163, 32-41.
Jouvet, M. and Michel, F., 1959. [Electromyographic correlations of sleep in the chronic decorticate & mesencephalic cat.]. C R Seances Soc Biol Fil. 153, 422-425.
Kandel, E. R., 2001. The molecular biology of memory storage: a dialogue between genes and synapses. Science. 294, 1030-1038.
Karni, A., Tanne, D., Rubenstein, B. S., Askenasy, J. J. and Sagi, D., 1994. Dependence on REM sleep of overnight improvement of a perceptual skill. Science. 265, 679-682.
Kattler, H., Dijk, D. J. and Borbély, A. A., 1994. Effect of unilateral somatosensory stimulation prior to sleep on the sleep EEG in humans. J Sleep Res. 3, 159-164.
Kaufman, L. S. and Morrison, A. R., 1981. Spontaneous and elicited PGO spikes in rats. Brain Res. 214, 61-72.
Kavanau, J. L., 1999. Adaptations and pathologies linked to dynamic stabilization of neural circuitry. Neurosci Biobehav Rev. 23, 635-648.
Kavushansky, A., Vouimba, R. M., Cohen, H. and Richter-Levin, G., 2006. Activity and plasticity in the CA1, the dentate gyrus, and the amygdala following controllable vs. uncontrollable water stress. Hippocampus. 16, 35-42.
Kim, E. Y., Mahmoud, G. S. and Grover, L. M., 2005a. REM sleep deprivation inhibits LTP in vivo in area CA1 of rat hippocampus. Neurosci Lett. 388, 163-167.
Kim, J. J., Koo, J. W., Lee, H. J. and Han, J. S., 2005b. Amygdalar inactivation blocks stress-induced impairments in hippocampal long-term potentiation and spatial memory. J Neurosci. 25, 1532-1539.

King, C., Recce, M. and O'Keefe, J., 1998. The rhythmicity of cells of the medial septum/diagonal band of Broca in the awake freely moving rat: relationships with behaviour and hippocampal theta. Eur J Neurosci. 10, 464-477.
Kitahama, K., Valatx, J. L. and Jouvet, M., 1981. Paradoxical sleep deprivation and performance of an active avoidance task: impairment of c57BR mice and no effect in c57BL/6 mice. Physiol Behav. 27, 41-50.
Klenerova, V., Kaminsky, O., Sida, P., Krejci, I., Hlinak, Z. and Hynie, S., 2002. Impaired passive avoidance acquisition in Sprague-Dawley and Lewis rats after restraint and cold stress. Behav Brain Res. 136, 21-29.
Kohsaka, M., Fukuda, N., Honma, K., Honma, S. and Morita, N., 1992. Seasonality in human sleep. Experientia. 48, 231-233.
Krueger, J. M. and Obál, F., Jr., 1993. A neuronal group theory of sleep function. J Sleep Res. 2, 63-69.
Kuhlmann, M. T., Clemen, G. and Schlatt, S., 2003. Molting in the Djungarian hamster (Phodopus sungorus Pallas): seasonal or continuous process? J Exp Zoolog A Comp Exp Biol. 295, 160-171.
Larkin, J. E. and Heller, H. C., 1998. The disappearing slow wave activity of hibernators. Sleep Research Online. 1, 96-101.
Lee, M. G., Chrobak, J. J., Sik, A., Wiley, R. G. and Buzsaki, G., 1994. Hippocampal theta activity following selective lesion of the septal cholinergic system. Neuroscience. 62, 1033-1047.
Leresche, N., Lightowler, S., Soltesz, I., Jassik-Gerschenfeld, D. and Crunelli, V., 1991. Low-frequency oscillatory activities intrinsic to rat and cat thalamocortical cells. J Physiol. 441, 155-174.
Leung, L. W., 1984. Model of gradual phase shift of theta rhythm in the rat. J Neurophysiol. 52, 1051-1065.
Lincoln, G. A., Andersson, H. and Loudon, A., 2003. Clock genes in calendar cells as the basis of annual timekeeping in mammals--a unifying hypothesis. J Endocrinol. 179, 1-13.
Lincoln, G. A. and Clarke, I. J., 2002. Noradrenaline and dopamine regulation of prolactin secretion in sheep: role in prolactin homeostasis but not photoperiodism. J Neuroendocrinol. 14, 36-44.
Linden, E. R., Bern, D. and Fishbein, W., 1975. Retrograde amnesia: prolonging the fixation phase of memory consolidation by paradoxical sleep deprivation. Physiol Behav. 14, 409-412.
Luppi, P. H., Gervasoni, D., Boissard, R., Verret, L., Goutagny, R., Peyron, C., Salvert, D., Leger, L., Barbagli, B. and Fort, P., 2004. Brainstem structures responsible for paradoxical sleep onset and maintenance. Arch Ital Biol. 142, 397-411.
Lyamin, O. I., Mukhametov, L. M., Chetyrbok, I. S. and Vassiliev, A. V., 2002. Sleep and wakefulness in the southern sea lion. Behav Brain Res. 128, 129-138.
Lynch, M. A., 2004. Long-term potentiation and memory. Physiol Rev. 84, 87-136.
Maquet, P., Laureys, S., Peigneux, P., Fuchs, S., Petiau, C., Phillips, C., Aerts, J., Del Fiore, G., Degueldre, C., Meulemans, T., Luxen, A., Franck, G., Van Der Linden, M., Smith, C. and Cleeremans, A., 2000. Experience-dependent changes in cerebral activation during human REM sleep. Nat Neurosci. 3, 831-836.
Marks, C. A. and Wayner, M. J., 2005. Effects of sleep disruption on rat dentate granule cell LTP in vivo. Brain Res Bull. 66, 114-119.
Marti-Nicolovius, M., Portell-Cortes, I. and Morgado-Bernal, I., 1988. Improvement of shuttle-box avoidance following post-training treatment in paradoxical sleep deprivation platforms in rats. Physiol Behav. 43, 93-98.
Massimini, M. and Amzica, F., 2001. Extracellular calcium fluctuations and intracellular potentials in the cortex during the slow sleep oscillation. J Neurophysiol. 85, 1346-1350.
McCarley, R. W., 2004. Mechanisms and models of REM sleep control. Arch Ital Biol. 142, 429-467.
McCormick, D. A. and Pape, H. C., 1990. Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurones. J Physiol. 431, 291-318.

McDermott, C. M., LaHoste, G. J., Chen, C., Musto, A., Bazan, N. G. and Magee, J. C., 2003. Sleep deprivation causes behavioral, synaptic, and membrane excitability alterations in hippocampal neurons. J Neurosci. 23, 9687-9695.

McGaugh, J. L., 2000. Memory--a century of consolidation. Science. 287, 248-251.

Morgan, P. J., Webster, C. A., Mercer, J. G., Ross, A. W., Hazlerigg, D. G., MacLean, A. and Barrett, P., 1996. The ovine pars tuberalis secretes a factor(s) that regulates gene expression in both lactotropic and nonlactotropic pituitary cells. Endocrinology. 137, 4018-4026.

Mukhametov, L. M., Supin, A. Y. and Polyakova, I. G., 1977. Interhemispheric asymmetry of the electroencephalographic sleep patterns in dolphins. Brain Res. 134, 581-584.

Nadasdy, Z., Hirase, H., Czurko, A., Csicsvari, J. and Buzsaki, G., 1999. Replay and time compression of recurring spike sequences in the hippocampus. J Neurosci. 19, 9497-9507.

Oleksenko, A. I., Mukhametov, L. M., Polyakova, I. G., Supin, A. Y. and Kovalzon, V. M., 1992. Unihemispheric sleep deprivation in bottlenose dolphins. J Sleep Res. 1, 40-44.

Pagel, J., Pegram, V., Vaughn, S., Donaldson, P. and Bridgers, W., 1973. The relationship of REM sleep with learning and memory in mice. Behav Biol. 9, 383-388.

Palchykova, S., Crestani, F., Meerlo, P. and Tobler, I., 2006a. Sleep deprivation and daily torpor impair object recognition in Djungarian hamsters. Physiol Behav. 87, 144-153.

Palchykova, S., Deboer, T. and Tobler, I., 2002. Selective sleep deprivation after daily torpor in the Djungarian hamster. J Sleep Res. 11, 313-319.

Palchykova, S., Deboer, T. and Tobler, I., 2003. Seasonal aspects of sleep in the Djungarian hamster. BMC Neurosci. 4, 9.

Palchykova, S., Winsky-Sommerer, R., Meerlo, P., Durr, R. and Tobler, I., 2006b. Sleep deprivation impairs object recognition in mice. Neurobiol Learn Mem.

Pavlides, C. and Winson, J., 1989. Influences of hippocampal place cell firing in the awake state on the activity of these cells during subsequent sleep episodes. J Neurosci. 9, 2907-2918.

Petsche, H., Stumpf, C. and Gogolak, G., 1962. [The significance of the rabbit's septum as a relay station between the midbrain and the hippocampus. I. The control of hippocampus arousal activity by the septum cells.]. Electroencephalogr Clin Neurophysiol. 14, 202-211.

Pothion, S., Bizot, J. C., Trovero, F. and Belzung, C., 2004. Strain differences in sucrose preference and in the consequences of unpredictable chronic mild stress. Behav Brain Res. 155, 135-146.

Rattenborg, N. C., Lima, S. L. and Amlaner, C. J., 1999. Facultative control of avian unihemispheric sleep under the risk of predation. Behav Brain Res. 105, 163-172.

Ribeiro, S., Gervasoni, D., Soares, E. S., Zhou, Y., Lin, S. C., Pantoja, J., Lavine, M. and Nicolelis, M. A., 2004. Long-lasting novelty-induced neuronal reverberation during slow-wave sleep in multiple forebrain areas. PLoS Biol. 2, E24.

Ribeiro, S., Goyal, V., Mello, C. V. and Pavlides, C., 1999. Brain gene expression during REM sleep depends on prior waking experience. Learn Mem. 6, 500-508.

Ribeiro, S., Mello, C. V., Velho, T., Gardner, T. J., Jarvis, E. D. and Pavlides, C., 2002. Induction of hippocampal long-term potentiation during waking leads to increased extrahippocampal zif-268 expression during ensuing rapid-eye-movement sleep. J Neurosci. 22, 10914-10923.

Ribeiro, S. and Nicolelis, M. A., 2004. Reverberation, storage, and postsynaptic propagation of memories during sleep. Learn Mem. 11, 686-696.

Romcy-Pereira, R. and Pavlides, C., 2004. Distinct modulatory effects of sleep on the maintenance of hippocampal and medial prefrontal cortex LTP. Eur J Neurosci. 20, 3453-3462.

Roth, C., Achermann, P. and Borbely, A. A., 1999. Alpha activity in the human REM sleep EEG: topography and effect of REM sleep deprivation. Clin Neurophysiol. 110, 632-635.

Ruf, T. and Heldmaier, G., 1992. The Impact of Daily Torpor on Energy Requirements in the Djungarian hamster, *Phodopus sungorus*. Physiol Zool. 65, 994-1010.

Ruskin, D. N., Liu, C., Dunn, K. E., Bazan, N. G. and LaHoste, G. J., 2004. Sleep deprivation impairs hippocampus-mediated contextual learning but not amygdala-mediated cued learning in rats. Eur J Neurosci. 19, 3121-3124.

Sakai, K. and Crochet, S., 2004. Role of the locus coeruleus in the control of paradoxical sleep generation in the cat. Arch Ital Biol. 142, 421-427.
Sakai, K., Petitjean, F. and Jouvet, M., 1976. Effects of ponto-mesencephalic lesions and electrical stimulation upon PGO waves and EMPs in unanesthetized cats. Electroencephalogr Clin Neurophysiol. 41, 49-63.
Sanchez-Vives, M. V. and McCormick, D. A., 2000. Cellular and network mechanisms of rhythmic recurrent activity in neocortex. Nat Neurosci. 3, 1027-1034.
Schwartz, W. J., de la Iglesia, H. O., Zlomanczuk, P. and Illnerova, H., 2001. Encoding le quattro stagioni within the mammalian brain: photoperiodic orchestration through the suprachiasmatic nucleus. J Biol Rhythms. 16, 302-311.
Schwierin, B., Achermann, P., Deboer, T., Oleksenko, A., Borbély, A. A. and Tobler, I., 1999. Regional differences in the dynamics of the cortical EEG in the rat after sleep deprivation. Clin Neurophysiol. 110, 869-875.
Sei, H., Saitoh, D., Yamamoto, K., Morita, K. and Morita, Y., 2000. Differential effect of short-term REM sleep deprivation on NGF and BDNF protein levels in the rat brain. Brain Res. 877, 387-390.
Seigneur, J., Kroeger, D., Nita, D. A. and Amzica, F., 2005. Cholinergic Action on Cortical Glial Cells In Vivo. Cereb Cortex.
Shiromani, P., Gutwein, B. M. and Fishbein, W., 1979. Development of learning and memory in mice after brief paradoxical sleep deprivation. Physiol Behav. 22, 971-978.
Siegel, J. M., 2001. The REM sleep-memory consolidation hypothesis. Science. 294, 1058-1063.
Siegel, J. M., 2005a. Clues to the functions of mammalian sleep. Nature. 437, 1264-1271.
Siegel, J. M., 2005b. The incredible, shrinking sleep-learning connection. Behavioral and Brain Sciences. 28, 82-83.
Silva, R. H., Chehin, A. B., Kameda, S. R., Takatsu-Coleman, A. L., Abilio, V. C., Tufik, S. and Frussa-Filho, R., 2004. Effects of pre- or post-training paradoxical sleep deprivation on two animal models of learning and memory in mice. Neurobiol Learn Mem. 82, 90-98.
Silvestri, A. J., 2005. REM sleep deprivation affects extinction of cued but not contextual fear conditioning. Physiol Behav. 84, 343-349.
Smith, C., 1995. Sleep states and memory processes. Behav Brain Res. 69, 137-145.
Smith, C. and Kelly, G., 1988. Paradoxical sleep deprivation applied two days after end of training retards learning. Physiol Behav. 43, 213-216.
Smith, C. and Lapp, L., 1986. Prolonged increases in both PS and number of REMS following a shuttle avoidance task. Physiol Behav. 36, 1053-1057.
Smith, C. and Rose, G. M., 1996. Evidence for a paradoxical sleep window for place learning in the Morris water maze. Physiol Behav. 59, 93-97.
Smith, C., Young, J. and Young, W., 1980. Prolonged increases in paradoxical sleep during and after avoidance-task acquisition. Sleep. 3, 67-81.
Smith, C. T., Conway, J. M. and Rose, G. M., 1998. Brief paradoxical sleep deprivation impairs reference, but not working, memory in the radial arm maze task. Neurobiol Learn Mem. 69, 211-217.
Snapp, B. D. and Heller, H. C., 1981. Suppression of Metabolism During Hibernation in Ground- Squirrels (Citellus-Lateralis). Physiological Zoology. 54, 297-307.
Soltesz, I., Lightowler, S., Leresche, N., Jassik-Gerschenfeld, D., Pollard, C. E. and Crunelli, V., 1991. Two inward currents and the transformation of low-frequency oscillations of rat and cat thalamocortical cells. J Physiol. 441, 175-197.
Steriade, M., 2003. The corticothalamic system in sleep. Front Biosci. 8, d878-899.
Steriade, M., Amzica, F. and Nunez, A., 1993a. Cholinergic and noradrenergic modulation of the slow (approximately 0.3 Hz) oscillation in neocortical cells. J Neurophysiol. 70, 1385-1400.
Steriade, M., Deschenes, M., Domich, L. and Mulle, C., 1985. Abolition of spindle oscillations in thalamic neurons disconnected from nucleus reticularis thalami. J Neurophysiol. 54, 1473-1497.
Steriade, M., Domich, L., Oakson, G. and Deschenes, M., 1987. The deafferented reticular thalamic nucleus generates spindle rhythmicity. J Neurophysiol. 57, 260-273.
Steriade, M., Dossi, R. C. and Nunez, A., 1991. Network modulation of a slow intrinsic oscillation of cat thalamocortical neurons implicated in sleep delta waves: cortically

induced synchronization and brainstem cholinergic suppression. J Neurosci. 11, 3200-3217.
Steriade, M., Iosif, G. and Apostol, V., 1969. Responsiveness of thalamic and cortical motor relays during arousal and various stages of sleep. J Neurophysiol. 32, 251-265.
Steriade, M., McCormick, D. A. and Sejnowski, T. J., 1993b. Thalamocortical oscillations in the sleeping and aroused brain. Science. 262, 679-685.
Steriade, M., Nunez, A. and Amzica, F., 1993c. Intracellular analysis of relations between the slow (< 1 Hz) neocortical oscillation and other sleep rhythms of the electroencephalogram. J Neurosci. 13, 3266-3283.
Steriade, M., Nunez, A. and Amzica, F., 1993d. A novel slow (< 1 Hz) oscillation of neocortical neurons in vivo: depolarizing and hyperpolarizing components. J Neurosci. 13, 3252-3265.
Steriade, M., Timofeev, I. and Grenier, F., 2001. Natural waking and sleep states: a view from inside neocortical neurons. J Neurophysiol. 85, 1969-1985.
Steriade, M. and Wyzinski, P., 1972. Cortically elicited activities in thalamic reticularis neurons. Brain Res. 42, 514-520.
Stern, W. C., 1971. Acquisition impairments following rapid eye movement sleep deprivation in rats. Physiol Behav. 7, 345-352.
Stetson, M. H., Watson-Whitmyre, M. and Matt, K. S., 1977. Termination of photorefractoriness in golden hamsters-photoperiodic requirements. J Exp Zool. 202, 81-88.
Stewart, M. and Fox, S. E., 1989. Two populations of rhythmically bursting neurons in rat medial septum are revealed by atropine. J Neurophysiol. 61, 982-993.
Stewart, M. and Fox, S. E., 1990. Do septal neurons pace the hippocampal theta rhythm? Trends Neurosci. 13, 163-168.
Stickgold, R., 1998. Sleep: off-line memory reprocessing. Trends Cogn Sci. 2, 484-492.
Stickgold, R., 2005. Sleep-dependent memory consolidation. Nature. 437, 1272-1278.
Stirland, J. A., Johnston, J. D., Cagampang, F. R., Morgan, P. J., Castro, M. G., White, M. R., Davis, J. R. and Loudon, A. S., 2001. Photoperiodic regulation of prolactin gene expression in the Syrian hamster by a pars tuberalis-derived factor. J Neuroendocrinol. 13, 147-157.
Strijkstra, A. M. and Daan, S., 1997. Sleep during arousal episodes as a function of prior torpor duration in hibernating European ground squirrels. J Sleep Res. 6, 36-43.
Strijkstra, A. M. and Daan, S., 1998. Dissimilarity of slow-wave activity enhancement by torpor and sleep deprivation in a hibernator. Am J Physiol. 275, R1110-R1117.
Struve, M. F., Brisbois, J. N., James, R. A., Marshall, M. W. and Dorman, D. C., 2001. Neurotoxicological effects associated with short-term exposure of Sprague-Dawley rats to hydrogen sulfide. NeuroToxicology. 22, 375-385.
Su, C. L., Chen, C. H., Lu, H. Y. and Gean, P. W., 2004. The involvement of PTEN in sleep deprivation-induced memory impairment in rats. Mol Pharmacol. 66, 1340-1348.
Sumova, A., Bendova, Z., Sladek, M., Kovacikova, Z. and Illnerova, H., 2004. Seasonal molecular timekeeping within the rat circadian clock. Physiol Res. 53 Suppl 1, S167-176.
Tamarkin, L., Baird, C. J. and Almeida, O. F., 1985. Melatonin: a coordinating signal for mammalian reproduction? Science. 227, 714-720.
Terao, A., Wisor, J. P., Peyron, C., Apte-Deshpande, A., Wurts, S. W., Edgar, D. M. and Kilduff, T. S., 2005. Gene expression in the rat brain during sleep deprivation and recovery sleep: an Affymetrix GeneChip((R)) study. Neuroscience.
Timofeev, I., Contreras, D. and Steriade, M., 1996. Synaptic responsiveness of cortical and thalamic neurones during various phases of slow sleep oscillation in cat. J Physiol. 494 (Pt 1), 265-278.
Timofeev, I., Grenier, F., Bazhenov, M., Sejnowski, T. J. and Steriade, M., 2000a. Origin of slow cortical oscillations in deafferented cortical slabs. Cereb Cortex. 10, 1185-1199.
Timofeev, I., Grenier, F. and Steriade, M., 2000b. Impact of intrinsic properties and synaptic factors on the activity of neocortical networks in vivo. J Physiol Paris. 94, 343-355.
Timofeev, I., Grenier, F. and Steriade, M., 2001. Disfacilitation and active inhibition in the neocortex during the natural sleep-wake cycle: an intracellular study. Proc Natl Acad Sci U S A. 98, 1924-1929.
Timofeev, I. and Steriade, M., 1996. Low-frequency rhythms in the thalamus of intact-cortex and decorticated cats. J Neurophysiol. 76, 4152-4168.

Tobler, I., 1992. Behavioral sleep in the Asian elephant in captivity. Sleep. 15, 1-12.
Tobler, I., 1995. Is sleep fundamentally different between mammalian species? Behav Brain Res. 69, 35-41.
Tobler, I., 2005. Phylogeny of Sleep Regulation. In: Kryger, M. H. et al. (Eds.), Principles and practice of sleep medicine. Elsevier Saunders, Philadelphia, PA, pp. 72-81.
Tobler, I., Deboer, T. and Fischer, M., 1997. Sleep and sleep regulation in normal and prion protein-deficient mice. J Neurosci. 17, 1869-1879.
Tobler, I. and Franken, P., 1993. Sleep homeostasis in the guinea pig: similar response to sleep deprivation in the light and dark period. Neurosci Lett. 164, 105-108.
Tobler, I., Franken, P. and Scherschlicht, R., 1990. Sleep and EEG spectra in the rabbit under baseline conditions and following sleep deprivation. Physiol Behav. 48, 121-129.
Tobler, I. and Jaggi, K., 1987. Sleep and EEG spectra in the Syrian hamster (Mesocricetus auratus) under baseline conditions and following sleep deprivation. J Comp Physiol [A]. 161, 449-459.
Tobler, I. and Scherschlicht, R., 1990. Sleep and EEG slow-wave activity in the domestic cat: effect of sleep deprivation. Behav Brain Res. 37, 109-118.
Tononi, G. and Cirelli, C., 2001. Modulation of brain gene expression during sleep and wakefulness: a review of recent findings. Neuropsychopharmacology. 25, S28-35.
Tononi, G. and Cirelli, C., 2003. Sleep and synaptic homeostasis: a hypothesis. Brain Res Bull. 62, 143-150.
Tononi, G. and Cirelli, C., 2006. Sleep function and synaptic homeostasis. Sleep Med Rev. 10, 49-62.
Trachsel, L., Edgar, D. M. and Heller, H. C., 1991. Are ground squirrels sleep deprived during hibernation? Am J Physiol. 260, R1123-R1129.
Trachsel, L., Tobler, I. and Borbely, A. A., 1986. Sleep regulation in rats: effects of sleep deprivation, light, and circadian phase. Am J Physiol. 251, R1037-1044.
Trachsel, L., Tobler, I. and Borbely, A. A., 1988. Electroencephalogram analysis of non-rapid eye movement sleep in rats. Am J Physiol. 255, R27-37.
Tsukada, M., Namade, N., Wada, Y., Mogi, T., Kamiyama, S. and Koizumi, A., 1993. Energy restriction supresses microsomal Ca^{2+}-ATPase activities in various organs in C57BL/6 female mice in both euthermic and torpor states. Mechanisms of Ageing and Development. 68, 183-189.
Vanderwolf, C. H., 1969. Hippocampal electrical activity and voluntary movement in the rat. Electroencephalogr Clin Neurophysiol. 26, 407-418.
Venkatakrishna-Bhatt, H., Bures, J. and Buresova, O., 1978. Differential effect of paradoxical sleep deprivation on acquisition and retrieval of conditioned taste aversion in rats. Physiol Behav. 20, 101-107.
Vertes, R. P., 2004. Memory consolidation in sleep; dream or reality. Neuron. 44, 135-148.
Vertes, R. P., 2005. Sleep is for rest, waking consciousness is for learning and memory - of any kind. Behavioral and Brain Sciences. 28, 86-87.
Villablanca, J. and Salinas-Zeballos, M. E., 1972. Sleep-wakefulness, EEG and behavioral studies of chronic cats without the thalamus: the 'athalamic' cat. Arch Ital Biol. 110, 383-411.
Villablanca, J. R., 2004. Counterpointing the functional role of the forebrain and of the brainstem in the control of the sleep-waking system. J Sleep Res. 13, 179-208.
Vyazovskiy, V. V., Achermann, P., Borbely, A. A. and Tobler, I., 2004a. The dynamics of spindles and EEG slow-wave activity in NREM sleep in mice. Arch Ital Biol. 142, 511-523.
Vyazovskiy, V. V., Borbély, A. A. and Tobler, I., 2000. Unilateral vibrissae stimulation during waking induces interhemispheric EEG asymmetry during subsequent sleep in the rat. Journal of Sleep Research. 9, 367-371.
Vyazovskiy, V. V. and Tobler, I., 2005. Theta activity in the waking EEG is a marker of sleep propensity in the rat. Brain Res. 1050, 64-71.
Vyazovskiy, V. V., Welker, E., Fritschy, J. M. and Tobler, I., 2004b. Regional pattern of metabolic activation is reflected in the sleep EEG after sleep deprivation combined with unilateral whisker stimulation in mice. Eur J Neurosci. 20, 1363-1370.
Walker, J. M. and Berger, R. J., 1980. Sleep as an adaptation for energy conservation functionally related to hibernation and shallow torpor. Prog Brain Res. 53, 255-278.
Walker, J. M., Garber, A., Berger, R. J. and Heller, H. C., 1979. Sleep and estivation (shallow torpor): continuous processes of energy conservation. Science. 204, 1098-1100.

Walker, J. M., Haskell, E. H., Berger, R. J. and Heller, H. C., 1980. Hibernation and circannual rhythms of sleep. Physiol Zool. 53, 8-11.
Walker, M. P., 2005. A refined model of sleep and the time course of memory formation. Behav Brain Sci. 28, 51-64; discussion 64-104.
Walker, M. P. and Stickgold, R., 2005. Sleep, Memory, and Plasticity. Annu Rev Psychol.
Wang, J. H., van den Buuse, M., Tian, S. W. and Ma, Y. Y., 2003. Effect of paradoxical sleep deprivation and stress on passive avoidance behavior. Physiol Behav. 79, 591-596.
Wang, X. J., 2002. Pacemaker neurons for the theta rhythm and their synchronization in the septohippocampal reciprocal loop. J Neurophysiol. 87, 889-900.
Wehr, T. A., 1991. The durations of human melatonin secretion and sleep respond to changes in daylength (photoperiod). J Clin Endocrinol Metab. 73, 1276-1280.
Wehr, T. A., Moul, D. E., Barbato, G., Giesen, H. A., Seidel, J. A., Barker, C. and Bender, C., 1993. Conservation of photoperiod-responsive mechanisms in humans. Am J Physiol. 265, R846-R857.
Welsh, D. K., Richardson, G. S. and Dement, W. C., 1985. A circadian rhythm of hippocampal theta activity in the mouse. Physiol Behav. 35, 533-538.
Werth, E., Achermann, P. and Borbely, A. A., 1996. Brain topography of the human sleep EEG: antero-posterior shifts of spectral power. Neuroreport. 8, 123-127.
Werth, E., Achermann, P. and Borbely, A. A., 1997a. Fronto-occipital EEG power gradients in human sleep. J Sleep Res. 6, 102-112.
Werth, E., Achermann, P., Dijk, D. J. and Borbely, A. A., 1997b. Spindle frequency activity in the sleep EEG: individual differences and topographic distribution. Electroencephalogr Clin Neurophysiol. 103, 535-542.
Wilson, C. J. and Kawaguchi, Y., 1996. The origins of two-state spontaneous membrane potential fluctuations of neostriatal spiny neurons. J Neurosci. 16, 2397-2410.
Wilson, M. A. and McNaughton, B. L., 1994. Reactivation of hippocampal ensemble memories during sleep. Science. 265, 676-679.
Wirz-Justice, A., Wever, R. A. and Aschoff, J., 1984. Seasonality in freerunning circadian rhythms in man. Naturwissenschaften. 71, 316-319.
Wolf, O. T., 2003. HPA axis and memory. Best Pract Res Clin Endocrinol Metab. 17, 287-299.
Woodfill, C. J., Wayne, N. L., Moenter, S. M. and Karsch, F. J., 1994. Photoperiodic synchronization of a circannual reproductive rhythm in sheep: identification of season-specific time cues. Biol Reprod. 50, 965-976.
Youngblood, B. D., Smagin, G. N., Elkins, P. D., Ryan, D. H. and Harris, R. B., 1999. The effects of paradoxical sleep deprivation and valine on spatial learning and brain 5-HT metabolism. Physiol Behav. 67, 643-649.
Zepelin, H., Siegel, J. M. and Tobler, I., 2005. Mammalian Sleep. In: Kryger, M. H. et al. (Eds.), Principles and practice of sleep medicine. Elsevier Saunders, Philadelphia, PA, pp. 91-100.
Zhang, J., Kaasik, K., Blackburn, M. R. and Lee, C. C., 2006. Constant darkness is a circadian metabolic signal in mammals. Nature. 439, 340-343.

Curriculum Vitae

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Born on December 10, 1974
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Education

2001 - 2005	Immatriculated at the MNF, University of Zurich Ph.D. Thesis Supervising Committee: Prof. R. Wehner, Prof. I. Tobler, Prof. A.A. Borbély Title of Ph.D. Thesis: “Sleep regulation and relationship between sleep and memory in rodents”
1999 - 2000	State Medical University of Kharkov, Department of Medical Biology, Genetics and Parasitology Postgraduate education under the supervision of Prof. G. F. Zhegunov.
1992 – 1997	Graduation Biology Diploma (Degree of Master of Science and Teacher of Biology and Chemistry, with Distinction)
1992 – 1997	State University of Kharkov, Faculty of Biology, Department of Animal and Human Physiology A diploma project: “Children's psychophysiological peculiarities in the process of learning under the implementing of different studying approaches”
1992	Certificate of secondary education
1982 – 1992	Secondary State School in Kharkov, Ukraine

Scientific experience

2000 – 2001	Ph.D. work at the Institute of Pharmacology and Toxicology, Section of Psychopharmacology and Sleep Research, University of Zurich, Switzerland Supervisor: Prof. I. Tobler

Award

2002	Poster award at the 16th Congress of the European Sleep Research Society, Reykjavik

Publications

Peer-reviewed publications

1. Palchykova S., Deboer T. and Tobler I. *Selective sleep deprivation after daily torpor in the Djungarian hamster.* J Sleep Res 2002, 11 (4): 313-319.
2. Palchykova S., Deboer T. and Tobler I. *Selasonal aspects of sleep in the Djungarian hamster.* BMC Neurosci 2003, 4-9.
3. Palchykova S., Crestani F., Meerlo P. Ad Tobler I. *Sleep deprivation and daily torpor impair object recognition in Djungarian hamsters.* Phisiol Behav 2006, 87(1): 144-153.
4. Palchykova S. and Tobler I. *Sleep, torpor and memory impairment.* JBIS 2006, 59 (3/4): 134-138.
5. Palchykova S., Winsky-Sommerer R., Meerlo P., Duerr R. and Tobler I. *Sleep deprivation impairs object recognition in mice.* Neurobiol Learn Mem 2006, 85(3): 263-271.

Abstracts and posters at International Congresses

1. Palchykova S., Deboer T. and Tobler I. *Homeostatic regulation of sleep after daily torpor in Djungarian hamsters.* J Sleep Res 2002, 11 (1): 168-169. 16th Congress of the European Sleep Research Society, Reykjavik, 2002.
2. Palchykova S., Kopp C. and Tobler I. *Daily torpor and sleep deprivation in the Djungarian hamster affect performance.* J Sleep Res 2004, 13 (s1): 548. 17th Congress of the European Sleep Research Society, Prague, 2004.
3. Vyazovskiy V.V., Palchykova S., Vesely M. and Tobler I. Rest consolidation as a measure of sleep homeostasis in mice. Society for Neuroscience (SfN) 35th Annual Meeting, 2005

Abstracts and posters at National Congresses

1. Palchykova S., Deboer T. and Tobler I. *Homeostatic regulation of sleep after daily torpor in the Djungarian hamster.* Neuroscience Center Zurich (ZNZ) Symposium, 2001
2. Palchykova S., Deboer T. and Tobler I. *Homeostatic regulation of sleep after daily torpor in the Djungarian hamster.* Swiss Society of Sleep Research, Sleep Medicine and Chronobiology (SGSSC) Meeting, 2001
3. Palchykova S. and Tobler I. *Seasonality of sleep in the Djungarian hamster.* ZNZ Symposium, 2002
4. Palchykova S. and Tobler I. *Seasonality of sleep in the Djungarian hamster.* Swiss Society for Neuroscience (SSN) Meeting, 2003
5. Palchykova S., Kopp C. and Tobler I. *Effects of daily torpor on recognition memory in the Djungarian hamster.* ZNZ Symposium, 2003
6. Palchykova S., Kopp C., Crestani F. and Tobler I. *Daily torpor decreased performance in an object recognition task in the Djungarian hamster.* Swiss Society of Sleep Research, Sleep Medicine and Chronobiology (SGSSC) Meeting, 2003

7. Palchykova S., Kopp C., Crestani F. and Tobler I. *Does daily torpor influence recognition memory in the Djungarian hamster?* Swiss Society for Neuroscience (SSN) Meeting, 2004
8. Palchykova S., Meerlo P., Crestani F. and Tobler I. Sleep deprivation impairs memory in Djungarian hamsters and mice. ZNZ Symposium, 2004
9. Palchykova S., Crestani F., Meerlo P. and Tobler I. *Sleep and memory in rodents.* Swiss Society for Neuroscience (SSN) Meeting, 2005
10. Palchykova S., Winsky-Sommerer R., Meerlo P., Dürr R. and Tobler I. *Sleep deprivation impairs recognition memory in mice.* Center for Integrative Human Physiology (CIHP) Opening Symposium, 2005
11. Palchykova S., Winsky-Sommerer R., Meerlo P., Dürr R. and Tobler I. *Sleep and recognition memory in mice.* ZNZ Symposium, 2005
12. Palchykova S., Winsky-Sommerer R., Meerlo P., Dürr R. and Tobler I. *Early, but not delayed sleep deprivation impairs object recognition memory in mice.* Swiss Society for Neuroscience (SSN) Meeting, 2006

List of abbreviations

ACTH	Adrenocorticotropic hormone
AMP	Adenosinemonophosphat
ANOVA	Analysis of variance
BDNF	Brain-derived neurotrophic factor
BL	Baseline
EEG	Electroencephalogram
EMG	Electromyogram
EPSP	Excitatory postsynaptic potential
ERK	Extracellular signal-regulated kinase
FFT	Fast Fourier Transform
HPA	Hypothalamo-pituitary adrenal
ICER	Inducible cyclic AMP early repressor
i.p.	Intraperitonial
IPSP	Inhibitory postsynaptic potential
LD	Light-dark
LTP	Long-term potentiation
mRNA	Messenger ribonucleic acid
MS-DBB	Medial septum and diagonal band of Broca area
NREM sleep	Non-rapid eye movement sleep
NSD	Partial non-rapid eye movement sleep deprivation
PGO	Ponto-geniculo-occipital
rANOVA	Repeated analysis of variance
RE	Thalamic reticular neurons
REM sleep	Rapid eye movement sleep
SCN	Suprachiasmatic nucleus
SD	Sleep deprivation
SE	Standard error of the mean
SWA	Slow-wave activity
SWE	Slow-wave energy
T_A	Ambient temperature
T_{body}	Body temperature
TC	Thalamocortical neurons
T_{CRT}	Cortical temperature
TST	Total sleep time